GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Volume 27 JULY, 1942 Number 4

TABLE OF CONTENTS

WRIGHT, SEWALL, TH. DOBZHANSKY, and W. HOVANITZ, Genetics of natural populations. VII. The allelism of lethals in the third chromosome of *Drosophila pseudoobscura* . . . 363

RHOADES, M. M., Preferential segregation in maize . . . 395

MOORE, JOHN A., An embryological and genetical study of *Rana burnsi* Weed 408

CLANCY, C. W., The development of eye colors in *Drosophila melanogaster*. Further studies on the mutant claret . . 417

LINCOLN, RALPH E., and JOHN W. GOWEN, Mutation of *Phytomonas stewartii* by X-ray irradiation 441

PUBLISHED BIMONTHLY AT MENASHA, WISCONSIN
BY THE BROOKLYN BOTANIC GARDEN
BROOKLYN, NEW YORK, U.S.A.

(Date of issue, July 20, 1942)

EDITORIAL BOARD

M. M. RHOADES, *Managing Editor*, Columbia University

WILLIAM E. CASTLE
University of California

LEON J. COLE
University of Wisconsin

EDWIN G. CONKLIN
Princeton University

CHARLES B. DAVENPORT
Carnegie Institution of Washington

BRADLEY M. DAVIS
University of Michigan

L. C. DUNN
Columbia University

ROLLINS A. EMERSON
Cornell University

JOHN W. GOWEN
Iowa State College

HERBERT S. JENNINGS
University of California, Los Angeles

DONALD F. JONES
Connecticut Agricultural Experiment Station

THOMAS H. MORGAN
California Institute of Technology

GEORGE H. SHULL
Princeton University

L. J. STADLER
U. S. Dept. Agriculture
University of Missouri

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Chicago

C. STUART GAGER, BUSINESS MANAGER, BROOKLYN BOTANIC GARDEN

Volume 27 JULY, 1942 Number 4

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $6.00 net a year for complete volumes (January–November). Parts of volumes are to be had only at the single number rate. Foreign postage, 60 cents additional. Single copies, $1.25 each, postpaid.

Back volumes, as available, may be had at $7.00 each, plus postage. The Business Manager will supply information on request as to odd volumes and numbers and complete sets available.

Correspondence concerning editorial matters should be addressed to the EDITOR OF GENETICS, 704 Schermerhorn Hall, Columbia University, New York.

Business Correspondence, including change of address, directions concerning reprints, and exchanges, should be addressed to GENETICS, Menasha, Wisconsin, U.S.A., or BROOKLYN BOTANIC GARDEN, 1000 Washington Ave., Brooklyn, N.Y., U.S.A.

Remittances should be made payable to GENETICS.

Entered as second-class matter, August 31, 1922, at the post-office at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L. & R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

GENETICS OF NATURAL POPULATIONS. VII. THE ALLELISM OF LETHALS IN THE THIRD CHROMOSOME OF DROSOPHILA PSEUDOOBSCURA

SEWALL WRIGHT, TH. DOBZHANSKY, AND W. HOVANITZ[1]
University of Chicago, Chicago; Columbia University, New York; and California Institute of Technology, Pasadena

Received November 25, 1941

INTRODUCTION

HETEROZYGOSIS for recessive mutant genes, some of which are lethal or semilethal in homozygotes, is very common in natural populations of Drosophila. In *D. pseudoobscura*, from fifteen percent (in the Death Valley region of California and Nevada) to thirty percent (in Mexico and Guatemala) of the third chromosomes contain lethals or semilethals. DOBZHANSKY and WRIGHT (1941) have reported that the frequency of alleles among lethals found in the population of a given mountain range in the Death Valley region is greater than that among lethals derived from populations of different mountain ranges. The importance of this observation lies in the fact that it permits certain inferences to be drawn regarding the breeding structure of natural populations of *D. pseudoobscura*. Further data bearing on the same problem are given in the present article.

The work to be described in the following pages has been made possible by a grant from the CARNEGIE INSTITUTION of WASHINGTON. The authors wish to express their appreciation to that Institution, and also to MRS. MARIAN GIBBS-HOVANITZ, MR. K. J. MAMPELL, and MR. ALEXANDER SOKOLOFF who have helped in conducting the experiments. Acknowledgement is also made to the DR. WALLACE C. and CLARA A. ABBOTT MEMORIAL FUND of the UNIVERSITY of CHICAGO for assistance in connection with the calculations.

MATERIAL

Most of the samples of natural populations of *Drosophila pseudoobscura* which served as material for the present study were collected in 1939 in three localities on the slopes of Mount San Jacinto, California. These localities are (fig. 1): Keen Camp (this locality is shown on the U. S. topographic maps as Hemet Valley; it is referred to below as "Keen"), Piñon Flats (later referred to as "Pinon"), and Andreas Canyon ("Andreas").

Keen is an almond shaped valley with a marshy meadow in the center draining into a semi-artificial Hemet Lake. The meadow lies at an elevation of slightly more than 4300 feet above sea level; it is surrounded by

[1] Observational and experimental data by TH. DOBZHANSKY and W. HOVANITZ, mathematical analysis by SEWALL WRIGHT.

GENETICS 27: 363 July 1942 (Date of issue, July 20, 1942).

Second Printing 1966 / University of Texas Printing Division, Austin

forest and brushland consisting chiefly of *Pinus ponderosa Jeffreyi*, *Quercus dumosa*, *Q. Kelloggii*, *Arctostaphylos glandulosa*, *Ceanothus cuneatus*, *Rhamnus californica*, *Adenostema sparsifolium*, *A. fasciculatum*, and *Artemisia tridentata*. The winters are long (freezing temperatures may be expected from November through April) and the rainfall relatively plentiful (normally 15 to 20 inches). The elevation of Pinon is approximately 4000 feet. Owing to a difference in soil and to Pinon being exposed to the hot and dry

FIGURE 1.—Map showing the location of the collecting stations on Mount San Jacinto.

winds from the Coachella and Imperial deserts, the ecological conditions are very different at Pinon and at Keen. The larger and most characteristic plants at Pinon are *Pinus cembroides monophylla*, *Juniperus californica*, *Arctostaphylos glauca*, *Condalia Parryi*, *Bernardia myricaefolia*, *Rhus trilobata*, *R. ovata*, *Purshia glandulosa* and *Quercus dumosa*. Andreas is a narrow canyon with a permanent stream rising in the upper reaches of San Jacinto and disappearing on the desert not far from the collecting locality, which

lies at an elevation of about 800 feet. The characteristic flora of the canyon bottom is *Populus Fremonti*, *Alnus rhombifolia*, *Washingtonia filifera*, *Platanus racemosa*, *Prunus eriogyna*, *Salix* sp., *Pluchea sericea*, *Prosopis chilensis*, *Acacia Greggii*, *Vitis Girdiana*, and others.[2]

Pinon is about 13 miles air line distant from either Keen and Andreas, while the distance between Andreas and Keen is about ten miles. Almost the entire territory shown in figure 1 which lies at 3500 or more feet above sea level is covered with trees or shrubs and therefore supports large and permanent populations of *D. pseudoobscura*. The ribbon of trees along the bottom of Andreas Canyon continues upward without major interruptions and eventually joins the mountain forest of San Jacinto. All the collecting localities described above consequently lie within a continuously populated region.

Nine collecting stations were chosen in the three localities; they are designated by letters following the locality name. Each "station" is a territory of at most 100 meters in diameter, on which 15 collecting bottles with fermenting banana were placed on previously marked trees or bushes. At Keen B, C, D, and E, and Pinon A and B the bottles were arranged more or less on the periphery of a circle. At Keen A and Andreas A and B owing to the local topography, the bottles were arranged in an irregular line from 50 (Andreas) to 100 (Keen A) meters long. The distances between the stations within a locality vary from about 200 meters (between Andreas A and B) to about 3.5 kilometers (between Keen C and E). Owing to the local peculiarities of the terrain, it is very difficult to estimate the areas of our "stations" and "localities"—that is, the areas for which our collections may be considered representative samples. The attractive radius of a banana trap bottle is also unknown, although certain unpublished experiments conducted in the summer of 1941 indicate that it cannot be much larger than 50 meters. As very rough approximations, we believe that the "stations" at Keen and Pinon are about 10,000 square meters, and at Andreas 5000 square meters or less. The area of the Keen "locality" is probably in the neighborhood of six square kilometers, that of the Pinon locality about 0.3 square kilometers, and that of the Andreas locality less than 50,000 square meters.

The trap bottles were exposed for a few hours in the late afternoon and at sunset, or else during the early morning hours. Occasionally, especially in winter at Andreas Canyon, flies may be procured during the day. In making repeated samplings of the same collecting station, the bottles were exposed, of course, always on the same trees.

A few of the flies included in the material of the present study came not

[2] The writers are indebted to DRS. H. BONNER, J. BONNER, and C. C. EPLING for the above lists of the characteristic plant species.

from Mount San Jacinto but from Wildrose Canyon, Panamint Mountains, California. Panamint is about 200 miles distant from San Jacinto and separated from the latter by a wide desert area. The population of Wildrose was sampled in 1937 (DOBZHANSKY and QUEAL 1938a) and in 1938 (KOLLER 1939). Two collecting stations, Wildrose A and B, were established there in 1939. Wildrose A corresponds almost exactly to the territory from which KOLLER'S sample was derived. DOBZHANSKY'S 1937 sample came from a larger territory, embracing Wildrose A, Wildrose B, as well as a distance of about 200 meters intervening between the two.

THE SEASONAL CYCLES IN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

Populations of *Drosophila pseudoobscura* behave differently in the several localities described above. At Keen, *D. pseudoobscura* is by far the commonest species of Drosophila, and its population at the height of the season must be very large. The flies appear early in April, become very abundant in June and early July, decline in numbers during July and August (especially at Keen C, which is the driest of the Keen collecting stations), remain at about the same level in September, and ostensibly disappear with the advent of cold weather in October or November. Although nothing is known about the refuges in which the flies spend the winters, the hibernating population at Keen is probably rather large. At Pinon, *D. pseudoobscura* is the commonest species of the genus, followed by *D. simulans* and *D. hydei*. It is abundant from March to May, rare during the hot and dry period of the summer, and again common from September to October or November (depending upon the occurrence of rain at that time). It is not in evidence during the winter months, although it seems possible that some individuals may be active on exceptionally warm days. At Andreas, *D. pseudoobscura*, *D. simulans*, *D. hydei*, and *D. victoria* become the most frequent species at different times of the year. *D. pseudoobscura* reaches its maximum abundance from January to March, declines thereafter, disappears almost completely from June to August, and gradually builds up its numbers from September to January. It is certain that its population is not destroyed entirely during the hot part of the summer, since single individuals may occasionally be trapped even in mid July. However, the population density must be very low in summer. The cycle at Wildrose is insufficiently known and is probably variable from year to year in this region of erratic rainfall. In two years the population was very dense in early June but scarce in September. Owing to the high elevation (close to 7200 feet), the winters are long and severe; how large is the hibernating population may only be conjectured.

DETECTION OF LETHALS AND SEMILETHALS IN THE THIRD CHROMOSOME

To detect the recessive mutant genes in the third chromosome, wild males or single sons of wild females are crossed individually to females homozygous for orange and purple (*or pr*). Single F_1 males are crossed to females carrying orange, Blade, Scute, and purple in one of their third chromosomes and an inversion and a lethal in the other (*or Bl Sc pr/C l*). In the next generation, flies showing *Bl* and *Sc* but not *or* and *pr* are selected and intercrossed; among the progeny, 33.3 percent of the zygotes are expected to be homozygous for the wild third chromosomes. The details of this technique have been described by DOBZHANSKY and QUEAL (1938b), and only certain features need be mentioned here. Provided a wild third chromosome carries no genetic factors affecting the viability, the progeny of the *Bl Sc* flies will consist of 33.3 percent wild type and 66.7 percent *Bl Sc* individuals. If the wild third chromosome carries a lethal, no wild types appear. Genes reducing the viability cause the frequency of wild types to fall between 0 and 33.3 percent. Such genes are very common in natural populations (DOBZHANSKY and QUEAL 1938b, DOBZHANSKY 1939). Those which give more than 0 but less than 16.5 percent of wild type flies in the cultures are arbitrarily designated semilethals. However, since cultures with from 10 to 20 percent of wild types are relatively rare, this arbitrariness introduces no serious error in our estimates of the frequencies of semilethals in nature.

Since the purpose of the present experiments has been to detect only the lethals and semilethals, but not the minor viability modifiers, no complete counts were made in a majority of the cultures. Cultures were examined when at least 50 to 100 flies were hatched in each of them; if a quarter or more of the flies were wild type, the culture was considered free of lethals or semilethals. Cultures in which few or no wild types were present on the first count were preserved, and further hatches were counted to determine whether the original deviation was due to the presence of lethals or semilethals, or to chance. We are confident that where the wild third chromosomes had gene arrangements other than the Standard, no lethals or semilethals escaped detection (these gene arrangements are mostly Arrowhead and Chiricahua I, occasionally Tree Line and Santa Cruz; for description of them see DOBZHANSKY and STURTEVANT 1938). The fact that some of the wild chromosomes in the populations studied had the Standard arrangement introduces a complication. Since the gene arrangement in the *or Bl Sc pr* chromosome is Standard, crossing over is free to take place in the *or Bl Sc pr*/ Standard wild females. A semilethal which lies in the chromosome far from the loci of the dominant marking genes *Blade* and *Scute* may be missed. Furthermore, in the chromosomes with the Standard gene arrangement the lethals are distinguishable from the semilethals chiefly

TABLE 1

Numbers of the third chromosome lethals and semilethals in samples of natural populations of Drosophila pseudoobscura. The figures in parentheses indicate the numbers of the chromosomes having the Standard gene arrangement.

COLLECTING STATION	DATE OF COLLECTING	LETHALS	SEMI-LETHALS	CHROMOSOMES EXAMINED
Keen A	April 25	1 (0)	—	7 (1)
	May 15	3 (2)	—	21 (8)
	June 20–21	9 (1)	5 (1)	67 (7)
	July 25–26	5 (2)	4 (2)	54 (18)
	Aug. 31–Sept. 1	1 (0)	—	14 (0)
	October 22	1 (0)	—	6 (1)
	Total	20 (5)	9 (3)	169 (35)
Keen B	April 25	—	1 (1)	6 (2)
	May 15	—	—	4 (2)
	May 20	3 (0)	—	24 (6)
	June 20–21	5 (0)	1 (0)	28 (4)
	June 29–30	2 (1)	1 (0)	21 (5)
	July 26–27	4 (1)	—	38 (8)
	Aug. 31–Sept. 1	—	1 (0)	18 (2)
	Total	14 (2)	4 (1)	139 (29)
Keen C	May 20	4 (2)	—	18 (4)
	June 2	8 (2)	—	37 (4)
	June 29–30	3 (0)	2 (0)	43 (2)
	July 25–26	1 (0)	—	15 (3)
	Sept. 1–2	1 (0)	—	7 (1)
	Total	17 (4)	2 (0)	120 (14)
Keen D	May 21	—	—	4 (0)
	June 3	6 (2)	1 (0)	62 (10)
	July 14–15	10 (2)	3 (0)	53 (14)
	Sept. 1–2	6 (0)	1 (0)	30 (5)
	Total	22 (4)	5 (0)	149 (29)
Keen E	May 22	1 (0)	—	3 (1)
	June 3	4 (2)	—	61 (15)
	July 14–15	3 (0)	2 (0)	40 (8)
	Aug. 18–19	1 (0)	—	26 (6)
	Total	9 (2)	2 (0)	130 (30)
Keen	Grand Total	82 (17)	22 (4)	707 (137)

COLLECTING STATION	DATE OF COLLECTING	LETHALS	SEMI-LETHALS	CHROMOSOMES EXAMINED
Andreas A	April 24	1 (0)	—	54 (28)
	May 13	1 (0)	1 (0)	17 (9)
	June 4	2 (0)	1 (0)	5 (0)
	Sept. 21	10 (4)	1 (1)	73 (37)
	Oct. 28–29	7 (1)	2 (0)	59 (21)
	Dec. 9	5 (4)	—	42 (22)
	March 2	8 (4)	2 (1)	93 (43)
	Total	34 (13)	7 (2)	343 (160)
Andreas B	April 24	—	1 (1)	19 (9)
	May 13	1 (1)	—	20 (10)
	June 4	3 (0)	—	19 (4)
	Sept. 21	9 (4)	3 (0)	103 (45)
	Oct. 28–29	9 (3)	—	47 (13)
	Dec. 9	3 (1)	—	23 (10)
	March 2	7 (3)	4 (0)	86 (40)
	Total	32 (12)	8 (1)	317 (131)
Andreas	Grand Total	66 (25)	15 (3)	660 (291)
Pinon A	April 23	—	1 (0)	13 (6)
	May 14	12 (2)	1 (0)	81 (19)
	June 21–22	3 (1)	—	23 (9)
	August 19–20	3 (0)	2 (0)	35 (7)
	Sept. 19–21	6 (2)	2 (1)	72 (25)
	Oct. 21–22	10 (6)	1 (1)	72 (29)
	Total	34 (11)	7 (2)	296 (95)
Pinon B	April 23	—	—	6 (6)
	May 14	6 (1)	3 (1)	64 (16)
	June 21–22	1 (0)	—	7 (2)
	August 19–20	1 (0)	—	28 (9)
	Sept. 19–21	5 (1)	2 (1)	57 (21)
	Oct. 21–22	1 (1)	1 (0)	69 (28)
	Total	14 (3)	6 (2)	231 (82)*
Pinon	Grand Total	48 (14)	13 (4)	527 (174)
San Jacinto	Grand Total	196 (56)	50 (11)	1894 (602)
Wildrose A	June 10	9 (2)	6 (0)	102 (23)
Wildrose B	June 10	14 (3)	5 (0)	94 (23)
Wildrose	Total	23 (5)	11 (0)	196 (46)

* The calculations are based on 79 as the number of Standard chromosomes at Pinon B. The original data were not accessible to the authors when the discrepancy was noted in correction of proof. Whatever its location, the effect on the conclusions is negligible.

if the latter make the surviving homozygotes phenotypically abnormal (which is frequently, but not invariably, the case). If the homozygotes are normal, a semilethal in a Standard chromosome is either recorded as a complete lethal or not detected at all.

THE FREQUENCIES OF LETHALS IN WILD CHROMOSOMES

The data are summarized in table 1. In view of the technical complications in the case of the Standard gene arrangement, it is desirable to compare the outcomes of the observations on the Standard and the Non-Standard chromosomes. The last two columns of table 2 show the frequencies and their standard errors of the Standard gene arrangement in various populations. There are highly significant differences in this respect among the regions considered here: San Jacinto 31.8 ± 1.1 percent, Death Valley 18.0 ± 1.2 percent, and it may be added, Mexico and Guatemala 0 percent (DOBZHANSKY 1939). There are, indeed, highly significant differences among the localities on San Jacinto: Keen 19.4 ± 1.5 percent, Pinon 33.0 ± 2.0 percent, and Andreas 44.1 ± 1.9 percent ($n = 2$, $\chi^2 = 99.6$, probability of such differences arising from accidents of sampling negligible). The significance of differences of this type between populations of neighboring localities will be discussed in another publication.

TABLE 2

The frequencies of lethals and semi-lethals.

	NON-STANDARD							STANDARD							% STANDARD	
	NO. CHROM.	LETHALS NO.	%	SE	SEMILETHALS NO.	%	SE	NO. CHROM.	LETHALS NO.	%	SE	SEMILETHALS NO.	%	SE		
San Jacinto																
Keen	570	65	11.4	1.3	18	3.2	0.7	137	17	12.4	2.8	4	2.9	1.5	19.4	1.5
Pinon	353	34	9.6	1.6	9	2.5	0.8	174	14	8.0	2.1	4	2.3	1.1	33.0	2.0
Andreas	369	41	11.1	1.6	12	3.3	0.9	291	25	8.6	1.6	3	1.0	0.6	44.1	1.9
Total	1292	140	10.84	0.86	39	3.02	0.48	602	56	9.3	1.18	11	1.83	0.55	31.8	1.1
Death Valley																
1937	707	91	12.9	1.3	26	3.7	0.7	142	10	7.0	2.1	0	0		16.7	1.3
Wildrose	150	18	12.0	2.7	11	7.3	2.1	46	5	10.9	4.5	0	0		23.5	3.0
Total	857	109	12.72	1.14	37	4.32	0.69	188	15	7.98	1.98	0	0		18.0	1.2
Grand Total	2149	249	11.59	0.69	76	3.54	0.40	790	71	8.99	1.02	11	1.39	0.42		

As shown in table 2, the frequencies of lethals are higher in Non-Standard than in Standard chromosomes, both at San Jacinto and in Death Valley, although not significantly so. The difference may be considered as probably significant in the grand total ($P = 0.05$). The deficiency of semilethals in Standard chromosomes, on the other hand, is clearly significant in the data from Death Valley ($P = 0.004$) and in the grand total ($P = 0.002$). This deficiency is due probably to the above mentioned difficulty

in the detection of semilethals in Standard chromosomes. Table 3 shows the relative frequencies of semilethals and of complete lethals among the mutants detected in natural populations and among the mutants obtained in the laboratory. In Non-Standard chromosomes, the percentages of semilethals among all lethals do not differ significantly among the three regions considered, the average being 24.1 ± 2.3 percent ($n = 2$, $\chi^2 = 1.4$, $P = 0.5$). This average, moreover, does not differ significantly from the corresponding figure in laboratory lethal mutations, 21.5 ± 5.1 percent ($P = 0.7$). Since semilethals as a class may be less strongly selected against in heterozygotes than complete lethals, this similarity of the percentage in nature and in the laboratory contributes somewhat to the evidence that there has been no important differential elimination of lethal mutations in nature.

TABLE 3

Percentage of semilethals among the total lethals found in nature or occurring in the laboratory. The Standard and Non-Standard chromosomes are distinguished in the wild populations. The laboratory mutants include only Non-Standard chromosomes.

	NON-STANDARD				STANDARD			
	COMPLETE AND SEMI-LETHALS	SEMILETHALS			COMPLETE AND SEMI-LETHALS	SEMILETHALS		
		NO.	%	SE		NO.	%	SE
Death Valley	146	37	25.4	3.6	15	0	0	—
San Jacinto	179	39	21.8	3.1	67	11	16.4	4.5
South of U. S.	36	11	30.6	7.7	0	—	—	—
Total (in nature)	361	87	24.1	2.3	82	11	13.4	3.8
Laboratory mutants	65	14	21.5	5.1				

In the Standard chromosomes, relatively fewer semilethals are recorded (13.4 ± 3.8 percent) than in Non-Standard chromosomes (24.1 ± 2.3 percent as noted above). The difference appears to be significant ($P = 0.03$).

It is obvious that accurate comparisons cannot be made in data that include semilethals in Standard chromosomes. It would be fairly satisfactory to use the data for all chromosomes but to consider only complete lethals. However, the best estimates of lethal frequencies are probably those based on Non-Standard chromosomes alone but including semilethals as well as lethals. Henceforward in the present paper the expression "lethal" will be used to include the semilethals, "complete lethal" being used where none of the recessives survives.

Table 4 shows the frequency of lethals by region and locality in Non-Standard chromosomes and in all chromosomes. Confining attention to Non-Standard, there are clearly significant differences in lethal frequency

between either San Jacinto (13.9 ± 1.0 percent) or Death Valley (17.0 ± 1.3 percent) and the region south of the United States (30.0 ± 4.2 percent). The slightly higher frequency in Death Valley compared with San Jacinto is probably significant (P = 0.02). There are no significant differences, however, among the localities of the same region (for example, in San

TABLE 4

The frequency of lethals, including semilethals, by region and locality. The frequencies in Non-Standard chromosomes are given in addition to those in all chromosomes because of the greater reliability of Non-Standard than Standard.

	NON-STANDARD				TOTAL			
	CHROMOSOMES NO.	LETHAL	% LETHAL	SE	CHROMOSOMES NO.	LETHAL	% LETHAL	SE
San Jacinto								
Keen	570	83	14.6	1.5	707	104	14.7	1.3
Pinon	353	43	12.2	1.7	527	61	11.6	1.4
Andreas	369	53	14.4	1.8	660	81	12.3	1.3
Total	1292	179	13.85	0.97	1894	246	12.99	0.77
Death Valley								
1937	707	117	16.5	1.4	849	127	15.0	1.2
Wildrose	150	29	19.3	3.2	196	34	17.3	2.7
Total	857	146	17.04	1.28	1045	161	15.41	1.12
South of U. S.								
Mexico	82	23	28.0	5.0				
Guatemala	38	13	34.2	7.7				
Total	120	36	30.0	4.2				

TABLE 5

The frequencies of lethals, including semilethals and of Standard, in all tested chromosomes at the various stations and localities of San Jacinto and of Wildrose (1939).

	NO. TESTED	% LETHAL	% STAND.		NO. TESTED	% LETHAL	% STAND.
Keen A	169	17.2	20.7	Andreas A	343	12.0	46.6
B	139	12.9	20.9	B	317	12.6	41.3
C	120	15.8	11.7				
D	149	18.1	19.5	Total	660	12.3	44.1
E	130	8.5	23.1				
Total	707	14.7	19.4				
Pinon A	296	13.9	32.1	Wildrose A	102	14.7	22.5
B	231	8.7	34.2	B	94	20.2	24.5
Total	527	11.6	33.0	Total	196	17.3	23.5

Jacinto, $n = 2$, $\chi^2 = 1.1$, $P = 0.6$) in marked contrast with striking differences in the frequencies of various gene arrangements in the third chromosomes in those same localities.

Tables 5 and 6 break down the data from localities according to station and date of collection, respectively. For the purpose here, the slight inaccuracy brought by inclusion of the Standard chromosomes is probably more than compensated for by the increase in numbers by such inclusion. The data accordingly refer to all chromosomes.

The frequency of the Standard chromosome shows no significant differences among the stations of the same locality (total, $n = 7$, $\chi^2 = 8.3$, $P = 0.3$). There are the marked differences, however, between localities already

TABLE 6

The frequencies of lethals, including semilethals, and of the Standard arrangement, according to month of collection at three localities of San Jacinto.

	KEEN			PINON			ANDREAS		
	NO. TESTED	% LETHAL	% STAND.	NO. TESTED	% LETHAL	% STAND.	NO. TESTED	% LETHAL	% STAND.
April 1939	13	14.9	27.6	19	14.0	28.6	73	2.7	50.7
May 1939	74			145			37	14.8	37.7
June 1939	319	14.7	14.7	30	13.3	36.7	24		
July 1939	200	16.0	25.5						
Aug. 1939	95	11.9	14.9	63	9.5	25.4			
Sept. 1939	–			129	11.6	35.7	176	13.1	46.6
Oct. 1939	6			141	9.2	40.4	106	17.0	32.1
Dec. 1939							65	12.3	49.2
Mar. 1940							179	11.7	46.4
Total	707	14.7	19.4	527	11.6	33.0	660	12.3	44.1

referred to above. The possibility that this may be related to differences in conditions is suggested by significant differences in the frequency of Standard according to date of collection in the same locality (total, $n = 12$, $\chi^2 = 30.8$, $P = 0.003$). This is brought out more adequately by other more extensive data which will be presented elsewhere.

With respect to the frequency of lethals, there are not only no significant differences among stations of the same locality (total, $n = 7$, $\chi^2 = 11.2$, $P = 0.1$) or among localities as previously discussed but also none according to date of collection (total, $n = 12$, $\chi^2 = 11.9$, $P = 0.5$). Thus the percentage of lethals as a group appears to be rather stable within all subdivisions of a region, irrespective of very great diversity of conditions, and at all times of the year. That there is some factor, however, that can make a difference in the percentage of lethals is shown by the probably significant differ-

ence between Death Valley and San Jacinto and the highly significant difference between both of these and the Mexico-Guatemala region discussed above.

We shall use 13.85 ± 0.97 percent based on the Non-Standard chromosomes as the most reliable estimate of the frequency of lethal bearing third chromosomes at San Jacinto. The corresponding figure for the 1937 collections at Death Valley, 16.5 ± 1.4 percent, is probably preferable for the region in 1937 to the figure based on all chromosomes (15.0 ± 1.2 percent) which was used in the previous paper (DOBZHANSKY and WRIGHT 1941), but the difference is not great. The former figure will be used in this paper as a basis for comparative analysis.

ALLELISM OF LETHALS

In an indefinitely large random breeding population, there would be no appreciable chance that two lethals taken at random have had a common origin. However, they may prove to be alleles in a certain proportion of cases merely because of recurrent mutation in a finite number of loci. In populations of limited size, this basic chance of allelism is supplemented by that due to the chance of common origin. As brought out in the preceding paper, comparison of the frequencies of allelism of lethals from the same or different populations is among the most important sources of information on the breeding structure of the species.

The technique of testing the allelism of third-chromosome lethals and semilethals is, briefly, as follows. Lethal-bearing third chromosomes are perpetuated in the form of balanced stocks of the constitution: lethal/*or Bl Sc pr*. If stocks containing non-allelic lethals are intercrossed, the progeny consists of *Bl Sc* and wild-type flies in a ratio approaching 2:1; if the lethals are alleles, the progeny consists of *Bl Sc* flies only. For details consult DOBZHANSKY and WRIGHT 1941.

To test the allelism of a group of lethals, every lethal-bearing strain must be outcrossed to all other strains of the group; with k lethals this requires $k(k-1)/2$ crosses. Since as many as 246 lethals and semilethals have been recovered from the San Jacinto populations alone (table 1), the labor of testing them all is prohibitive. In view of this, we have performed all the possible intercrosses of the lethals and semilethals found in the samples from the same stations, while only a part of the intercrosses of lethals recovered from different stations and localities have been made.

The basic chance of allelism, due merely to recurrent mutation, may be determined by testing lethals from such remote populations that there is no appreciable chance of common origin.

The results of intercrosses of lethals from different localities on Mount San Jacinto and of lethals from Mount San Jacinto and Wildrose (Death

Valley region) are shown in table 7. The numbers of the lethals examined are shown in parentheses after the name of locality; thus, "Keen (15) ×Pinon (15)" signifies that fifteen lethals from Keen were intercrossed with fifteen lethals from Pinon; the number of the intercrosses necessary to test this group of lethals is evidently $15 \times 15 = 225$.

TABLE 7

Numbers of intercrosses made and numbers of alleles found among lethals from populations of different localities and regions.

LOCALITIES	NUMBER OF INTERCROSSES	NUMBER OF ALLELES
Keen (15)×Pinon (15)	225	1
Keen (15)×Andreas (15)	225	1
Pinon (16)×Andreas (16)	256	2
Keen (15)×Wildrose (15)	225	0
Pinon (15)×Wildrose (15)	225	1
Andreas (15)×Wildrose (15)	225	1
Total	1381	6

These data may be supplemented by the data published previously on the frequency of allelism of lethals from different mountain forests of Death Valley. Table 8 below gives a condensed summary of all the data bearing on this question.

TABLE 8

Frequency of allelism in tests of lethals, including semilethals, from different localities either of the same or different regions.

REGION	NO. TESTS	SAME LOCUS	%	SE
San Jacinto	706	4	0.57	0.28
Death Valley	4913	20	0.41	0.09
S.J. and D.V.	675	2	0.30	0.21
Total	6294	26	0.413	0.081

Taking the figures at face value, the percentage of allelism decreases with distance, but the differences are not significant ($n = 2$, $\chi^2 = 0.65$, $P = 0.7$). The grand average, 0.413 ± 0.081 percent will be used as an estimate of the chance of allelism at localities so remote that independent origin may be assumed.

At the opposite extreme is the chance of allelism within a station. The data for the intra-station crosses are presented in table 9. As explained

above, the number of crosses necessary to test the allelism, for example, of the 27 lethals of Keen A is 351. Table 1 however, shows that the 29 lethals, recovered from the population of this station (two of which were lost before the allelism tests were made) came from six samples taken at approximately monthly intervals from April to October 1939. Some of the 351 crosses consequently were between lethals collected simultaneously and

TABLE 9

Numbers of intercrosses made and numbers of alleles found among lethals from the populations of the same station.

STATION	LETHALS EXAMINED	COLLECTED SIMULTANEOUSLY		COLLECTED AT DIFFERENT TIMES		TOTAL	
		INTERCROSSES	ALLELES	INTERCROSSES	ALLELES	INTERCROSSES	ALLELES
Keen A	27	109	—	242	4	351	4
Keen B	17	25	1	111	2	136	3
Keen C	17	37	1	99	6	136	7
Keen D	25	108	2	192	7	300	9
Keen E	11	16	—	39	1	55	1
Pinon A	34	119	6	442	4	561	10
Pinon B	16	38	1	82	2	120	3
Andreas A	19	52	1	119	1	171	2
Andreas B (1939)	21	62	1	148	2	210	3
Andreas B (March 2, 1940)	8	28	2	—	—	28	2
Wildrose A (June 10, 1939)	15	105	—	—	—	105	—
Wildrose B (June 10, 1939)	13	78	1	—	—	78	1
Total	223	777	16	1474	29	2251	45

others between lethals from samples collected at different times. Since it is desirable to compare the frequency of alleles among lethals from the same sample with that among lethals collected at different times, the data in table 9 are differentiated accordingly.

Table 10 summarizes the results of the intercrosses between lethals found in the populations of different stations within a single locality. Since the population of every station (except Wildrose) has been sampled repeatedly during 1939, we may distinguish the intercrosses involving lethals detected in samples taken on the same day or within a short time interval (less than a month), and the intercrosses of lethals derived from samples taken at times from a month to several months apart. The former are shown in tables 9 and 10 in the columns labelled "Collected simultaneously," and the latter in the columns labelled "Collected at different times."

Table 11 gives a condensed summary of the data from San Jacinto according to whether the flies came from the same station or different stations of the same locality and according to whether they were collected simultaneously or not.

TABLE 10

Numbers of intercrosses made and numbers of alleles found among lethals from populations of different stations within a locality.

STATIONS	COLLECTED SIMULTANEOUSLY		COLLECTED AT DIFFERENT TIMES	
	NUMBER OF INTERCROSSES	NUMBER OF ALLELES	NUMBER OF INTERCROSSES	NUMBER OF ALLELES
Keen A×Keen B	112	2	180	0
Keen A×Keen C	21	0	156	3
Keen A×Keen D	96	2	178	2
Keen A×Keen E	3	0	68	0
Keen B×Keen C	36	1	78	0
Keen B×Keen D	3	0	89	0
Keen B×Keen E	3	0	9	0
Keen C×Keen D	56	1	86	0
Keen C×Keen E	36	0	22	0
Keen D×Keen E	100	0	37	0
Pinon A×Pinon B	126	2	398	5
Andreas A×Andreas B	99	1	292	1
Wildrose A×Wildrose B	230	2	—	—
Total	921	11	1593	11

In the earlier study of data from Death Valley, the tests for allelism of lethals from the same locality were based wholly on flies collected simultaneously from a few traps. It was pointed out that there was a possibility that the proportion of alleles might be higher than among random flies from

TABLE 11

Frequency of allelism of lethals (including semilethals) according to whether the flies were collected at the same or different stations of the same locality and according to whether collected simultaneously or at different times (San Jacinto only).

	SAME STATION				DIFFERENT STATIONS			
	NO. TESTS	SAME LOCUS	%	SE	NO. TESTS	SAME LOCUS	%	SE
Simultaneous	594	15	2.53	0.64	691	9	1.30	0.43
Different times	1474	29	1.97	0.36	1593	11	0.69	0.21
Total	2068	44	2.13	0.32	2284	20	0.88	0.20

the locality (DOBZHANSKY and WRIGHT 1941). In the present data, the percentage of alleles in tests of flies caught simultaneously (2.53 ± 0.64 percent is indeed slightly higher than in the tests of flies caught at the same station several months apart (1.97 ± 0.36 percent), but the difference is not significant ($P = 0.4$). The difference is in the same direction in the case of flies caught in different stations (simultaneously 1.30 ± 0.43 percent alleles, non simultaneously 0.69 ± 0.21 percent alleles), but again the difference is not significant ($P = 0.2$).

What is really wanted is the chance of allelism in a random sample of a single generation. This might be expected to be a little less than that from flies caught in a few traps but a little larger than that from flies caught over a period in which the frequencies of lethals have had an opportunity to change. In view of recent studies indicating very rapid diffusion of flies

TABLE 12

Frequency of allelism of lethals, including semilethals, according to locality and according to whether collected at the same or different stations of the same locality (same or different locality of the region is the case of Death Valley 1937).

LOCALITY	SAME STATION				DIFFERENT STATIONS			
	NO. TESTS	SAME LOCUS	%	SE	NO. TESTS	SAME LOCUS	%	SE
San Jacinto								
Keen	978	24	2.45	0.49	1369	11	0.80	0.24
Pinon	681	13	1.91	0.52	524	7	1.34	0.50
Andreas	409	7	1.71	0.64	391	2	0.51	0.36
Total	2068	44	2.13	0.32	2284	20	0.88	0.20
Death Valley 1937	772	24	3.11	0.63				
Wildrose 1939	183	1	0.55		230	2	0.87	0.67

from a point of release (unpublished), it has become doubtful whether the former consideration has much weight. Thus it is probable that the grand averages for stations and localities tend to be slightly too low rather than too high. This is especially the case with the figure for localities, which should, ideally, be based on allelism tests of a large number of lethals collected at random throughout the territory designated as "locality." In our computations the allelic lethals found within stations are entirely excluded from consideration as far as the frequency of allelism within the localities is concerned, although they should have some weight in completely random tests of a locality.

Table 12 gives a condensed summary of the data according to locality. The data from the 1937 collections at Death Valley are included under the

caption "same station," but the flies were taken from areas comparable to the Andreas and Pinon localities of San Jacinto.

There are no significant differences between the localities at San Jacinto in spite of the great differences in conditions (for intra-station tests $n=2$, $\chi^2=1.0$, $P=0.6$; for inter-station tests $n=2$, $\chi^2=1.9$, $P=0.4$). There is a great difference between the high percentage of allelism in Death Valley localities in 1937 (3.11 ± 0.63) and either the intra- or inter-station results at one locality, Wildrose, in 1939. The numbers at Wildrose were small, but the combined percentage (0.73 ± 0.32 percent) is significantly less than the earlier figure ($P=0.01$). It is possible that conditions differed markedly in 1937 and 1939.

Returning to the data from San Jacinto, the percentage of alleles among flies from the same station (2.13 ± 0.32 percent) is significantly greater ($P=0.0005$) than among flies from different stations of the same locality (0.88 ± 0.20 percent). This in turn is significantly greater ($P=0.009$) than among flies from different localities or regions (0.413 ± 0.081 percent). These are the figures that will be used in further analysis, but, as noted, the two former are probably somewhat too low for estimates of allelism of random flies from the same station or same locality in a single generation.

LETHALS OF COMMON ORIGIN

In testing the allelism of third-chromosome lethals from the Death Valley region, DOBZHANSKY and WRIGHT (1941) have observed that the lethals found repeatedly within the population of a station on an isolated mountain range are not significantly commoner in the populations of other ranges than are the lethals encountered within a station only once. Conversely, the lethals encountered on two or more mountain ranges show no tendency to accumulate in populations of any one range. This observation indicates that the allelic lethals found within the population of a small territory are frequently descendants of a single original mutant, while the allelism of lethals on different mountain ranges is due mainly to independent origin of similar mutants in two or more populations. To put it another way, the lethals found repeatedly within a population are not necessarily those which arise most frequently in the species at large.

Although not all the possible intercrosses between the lethals detected in Mount San Jacinto populations have been made, the material of the present study may be used to check the validity of the above observations. Table 13 shows the number of lethals found once ("singles"), twice ("doublets"), and three times ("triplets") in the populations of the stations examined.

Among the 152 lethals, each of which was found singly within a station (table 13), only 18, or 11.9 percent, were detected in populations of other

stations within the same locality, and 11, or 7.2 percent, were encountered in other localities or regions. Among the 25 doublets, seven, or 28.0 percent, were found at other stations within the locality, and none was found in other localities or regions. Finally, three out of the seven triplets, or 42.9 percent, were met with at other stations within the same locality, and one out of the seven, or 14.3 percent, in a different region. The same lethal was found thrice at Keen C, thrice at Keen D, and once at Keen A, but it was not encountered at all outside the Keen locality. Another lethal was found

TABLE 13

Number of lethals found once, twice, and thrice within the populations of separate stations.

STATION	SINGLES	DOUBLETS	TRIPLETS	STATION	SINGLES	DOUBLETS	TRIPLETS
Keen A	19	4	—	Pinon B	10	3	—
Keen B	11	3	—	Andreas A	15	2	—
Keen C	9	1	2	Andreas B (1939)	15	3	—
Keen D	13	3	2	Andreas B (1940)	4	2	—
Keen E	9	1	—	Wildrose A	15	—	—
Pinon A	21	2	3*	Wildrose B	11	1	—
				Total	152	25	7

* Although the lethals Pinon A 169 and Pinon A 170, as well as Pinon A 170 and Pinon A 429, proved to be alleles, the cross Pinon A 169×Pinon A 429 gave a result showing that these lethals are not alleles. As shown in the previous paper, an appreciable portion of the lethal chromosomes are expected to contain two or more lethal genes of independent origin. Pinon A 170 may have been of this sort. It could also, of course, have been a deficiency covering more than a single locus.

thrice at Pinon A, once at Pinon B, once at Andreas B, and once at Wildrose A, but it was not met with at Keen.

Despite the smallness of the above figures, the situation is clearly that lethals which have attained high frequencies within the population of a given station tend to be found in the populations of other stations within the same locality. This is the opposite of what has been found to be the case in the Death Valley region. The contradiction, however, is explicable if the characteristics of the terrain on Mount San Jacinto and in Death Valley are taken into account. All the collecting stations on Mount San Jacinto are in a territory which supports a dense and continuous population of *D. pseudoobscura*, while the desert gaps which lie athwart the possible migration paths between the mountain ranges of the Death Valley region are obstacles for the movement of the flies.[3] Furthermore, the absolute

[3] Deserts are certainly not absolute barriers for fly migration. A number of collectors (DOBZHANSKY, MAINLAND, MAMPELL, EPLING, and others) have repeatedly taken *D. pseudoobscura* amid desert vegetation, and in spring following a rainy winter they may not be even rare. Nevertheless, the habitat in which permanent, large, and flourishing populations of this species are

distances between the collecting stations on Mount San Jacinto are smaller than those between the ranges in the Death Valley region. A mutant gene which, by chance or otherwise, has attained a high frequency on a mountain range in Death Valley may not spread to the adjacent ranges for a long time, but there is no comparable handicap for the diffusion of genes from population to population within a locality on Mount San Jacinto. For an understanding of the population structure in *D. pseudoobscura*, however, it is important to know that a mutant which is lethal when homozygous may not only increase in frequency in the immediate vicinity of the place of its origin but may spread to populations within a mile or more from that place.

MUTATION RATE

One of the statistics that is needed in attempting to analyze the situation in nature is the rate of occurrence of lethal mutations of the third chromosome of *Drosophila pseudoobscura*. The available data are recapitulated below (DOBZHANSKY and WRIGHT 1941).

TABLE 14

Data on rate of occurrence of lethal mutations in the third chromosome of Drosophila pseudoobscura.

SOURCE OF STOCK	KIND OF EXPERIMENT	CHROMOSOMES TESTED NO.	LETHAL MUTANTS NO.	%	SE	AVERAGES %	AVERAGES SE
Death Valley	direct	3580	9	0.25	0.08	0.297	0.047
Death Valley	cumulative	9892	31	0.31	0.06		
Mexico	cumulative	4177	15	0.36	0.09	0.325	0.065
Guatemala	cumulative	3522	10	0.28	0.09		
Total		21171	65			0.307	0.038

The results from Death Valley (two experiments), Mexico, and Guatemala are in excellent agreement ($n=3$, $\chi^2=0.81$, $P=0.8$). In the study of the situation in Death Valley the figure used was the average mutation rate from Death Valley stock—namely, 0.297 ± 0.047 percent. Since the rate has not been obtained from stock from San Jacinto, the best available estimate for this region seems to be that given by the grand total in table 14—namely, 0.307 ± 0.038 percent. The difference from the figure used for Death Valley is of little importance.

Determinations for Death Valley were made in two ways: (1) a direct determination of mutation rate per generation and (2) a determination

regularly found is unquestionably connected with tree vegetation. The flies can move more freely within the forest-covered portions of the isolated mountain ranges in the Death Valley region (DOBZHANSKY and QUEAL 1938a, 1938b) than across the desert gaps between these ranges.

from experiments in which mutations were allowed to accumulate for several generations before examination. The mutation rate obtained from the latter method should be lower if an appreciable portion of the mutations are strongly selected against as heterozygotes. Since the result was actually higher (though not significantly so), it was concluded that there is no important immediate elimination of lethal mutations due to this cause.

ESTIMATES OF POPULATION CONSTANTS

As brought out in the previous paper (DOBZHANSKY and WRIGHT 1941) it requires a rather large number of constants to give even a very rough description of the genetic situation in a natural population. The description of the breeding structure requires at least three.

N. The effective size of population. Roughly the harmonic mean of the numbers of mating individuals per generation throughout the year and hence much more closely related to the minimum than to the maximum number.

F. The inbreeding coefficient. This measures the departure from random mating within the population in question. With gene frequency q the zygotic frequencies are

$$[(1-F)(1-q)^2+F(1-q)]AA+2(1-F)q(1-q)Aa+[(1-F)q^2+Fq]aa.$$

This is modified by selection but not much if the amount of selective elimination is small.

m. The migration index. This measures the extent to which there is replacement in each generation by immigrants drawn from a population sufficiently large that each lethal is at its equilibrium frequency.

There was some ambiguity in the symbols used in the earlier paper to represent the properties of the lethal genes. Changes are made here to avoid this. Each lethal gene in the given population has a certain frequency (q) resulting from its rate of origin by mutation (v), its rate of elimination in homozygotes, the selection against it when heterozygous (s), and the accumulation of random deviations due to the accidents of sampling. We assume that the homozygous semilethals leave so few descendants that they may be grouped with the complete lethals with no appreciable error.

With regard to the accidents of sampling, let q_i be the frequency of a lethal belonging to the class characterized by particular values of s and v —namely, s_i and v_i. In a small population the genes of this class should exhibit in the long run a distribution, $\phi(q_i)$, characterized by a certain mean, $\bar{q}_i$, and a certain variance $\sigma_{q_i}^2$. The actual mean frequency for all lethals ($\bar{q}$) should equal the mean of these class means except for second order fluctuations. The actual variance of the frequencies of all lethals,

σ_q^2 (σ_{qt}^2 in the previous paper) is analyzable into two components. One of these is the mean of the values of σ_{qi}^2 for all classes, which will be represented by $\sigma_{q(e)}^2$ (represented by σ_q^2 in the previous paper). The other is the variance of class means due to differences in s_i and v_i, which will be represented here by $\sigma_{q(d)}^2$ (represented by $\sigma_{\bar{q}}^2$ in the previous paper).

n Number of loci in third chromosome subject to lethal mutation.

$\bar{v}$ Mean mutation rate per generation per locus.

$\bar{s}$ Mean selective disadvantage of heterozygotes.

$\bar{q}$ Mean frequency of lethals.

σ_q^2 Variance of frequencies of lethals.

$\sigma_{q(e)}^2$ Component of σ_q^2 due to cumulative effects of accidents of sampling.

$\sigma_{q(d)}^2$ Component of σ_q^2 due to differences among lethals in v and s.

p Chance of allelism of two lethal genes taken at random from the specified population (p_{sta} from "stations," p_{loc} from "localities").

p_∞ Chance of allelism of two lethal genes taken at random from a population sufficiently large that each is at its equilibrium frequency.

The 12 constants listed above are not all independent of each other. Equation (1) below holds by definition, and equations (2) and (3) were demonstrated in the previous paper (p. 38). Equation (4) follows from the other three.

$$\sigma_q^2 = \sigma_{q(e)}^2 + \sigma_{q(d)}^2 \tag{1}$$

$$p = n(\bar{q}^2 + \sigma_q^2)/(n\bar{q})^2 \tag{2}$$

$$p_\infty = n(\bar{q}^2 + \sigma_{q(d)}^2)/(n\bar{q})^2 \tag{3}$$

$$n\sigma_{q(e)}^2 = (p - p_\infty)(n\bar{q})^2. \tag{4}$$

Other relations depend on whether v and s are assumed to be the same for all genes, to vary only moderately about their mean, or to vary greatly. As in the previous paper, we shall begin with the assumption that there is only moderate variation of v and s, if any.

None of the above quantities are observed directly. The principal observed quantities are as follows:

V The rate of lethal mutation per generation per *chromosome.*

Q The frequency of lethal bearing third *chromosomes.*

P The chance of allelism of two random lethal *chromosomes* from the specified population (P_{sta} from "stations," P_{loc} from "localities").

P_∞ The chance of allelism of two random lethal *chromosomes* from a population sufficiently large that independent mutant origin is reasonably certain.

The conditions at the three localities on San Jacinto are so different that it is desirable to attempt separate analysis, although the numbers are

hardly adequate except perhaps at Keen. Analysis will also be carried through for the average of these localities and will be compared with revised figures for Death Valley (1937 only) and as far as possible with those from Mexico and Guatemala. The frequencies in all cases are based on the chromosomes other than Standard, instead of on all chromosomes, as in the previous study. The collecting areas at Death Valley were made from areas roughly comparable to smaller localities, Pinon and Andreas, at San Jacinto. The following figures are those which will be used (see table 4, 8, 12, and 14).

	Q	P_{sta}	P_{loc}
San Jacinto			
Keen	0.146 ±0.015	0.0245±0.0049	0.0080±0.0024
Pinon	0.122 ±0.017	0.0191±0.0052	0.0134±0.0050
Andreas	0.144 ±0.018	0.0171±0.0064	0.0051±0.0036
Average	0.1385±0.0097	0.0213±0.0032	0.0088±0.0020
Death Valley	0.165 ±0.014	—	0.0311±0.063
Mexico and Guatemala	0.300 ±0.042	—	—

V=0.00307±0.00038 (based on stocks from Death Valley, Mexico, and Guatemala)
P_∞=0.00413±0.00081 (based largely on Death Valley localities but partly on San Jacinto)

These figures relating to lethal chromosomes must be translated as well as possible into the corresponding expressions relating to lethal genes. As before, it is assumed that independently occurring lethals are distributed in the chromosomes according to the terms of the Poisson series $\exp[-n\bar{q}(1-l)]$ where l symbolizes a lethal. The possibility of simultaneous occurrence of two or more lethals (as from deficiencies) is ignored. The frequency of chromosomes lacking all lethals is thus taken to be $(1-Q)=\exp(-n\bar{q})$

$$n\bar{q} = -\log_e(1-Q). \tag{5}$$

It was shown in the previous paper that the chance of allelism of lethal chromosomes drawn from remote populations must be multiplied by $[Q/n\bar{q}]^2$ to give that for lethal genes.

$$p_\infty = P_\infty[Q/n\bar{q}]^2. \tag{6}$$

Within small populations, lethal chromosomes that prove to be allelic are most likely to trace to a common source and to be alike in all lethal genes. Thus p was assumed to be equal to P in the previous paper. However, there should be the same chance of separate origin as in the case of remote populations, and a small correction $(P_\infty - p_\infty)$ should be made.

$$p_{sta} = P_{sta} - (P_\infty - p_\infty) \tag{7}$$

$$p_{loc} = P_{loc} - (P_\infty - p_\infty). \tag{8}$$

No correction is necessary to relate mutation rate per locus to that per chromosome.

$$n\bar{v} = V. \tag{9}$$

Application of (5) gives the estimates of $n\bar{q}$ given below. Substitution of the values of P_∞, Q and $n\bar{q}$, in (6) gives 0.00345 as the estimate of p_∞ from the Death Valley data and 0.00356 from that from San Jacinto. We shall use the average , $p_\infty = 0.0035$.

The values of p_{sta} and p_{loc} may now be obtained by subtracting 0.0006 $(=P_\infty - p_\infty)$ from the values of P_{sta} and P_{loc} (7, 8), respectively. These results permit estimates of $n\sigma_{q(c)}^2$ for stations and localities from the equation (4).

TABLE 15

Estimate of $n\bar{q}$ and of p and $n\sigma_{q(c)}^2$ for both stations and localities.

	$n\bar{q}$	STATION		LOCALITY	
		p_{sta}	$n\sigma_{q(c)}^2$	p_{loc}	$n\sigma_{q(c)}^2$
San Jacinto					
Keen	0.158	0.0239	0.000508	0.0074	0.000097
Pinon	0.130	0.0185	0.000254	0.0128	0.000157
Andreas	0.155	0.0165	0.000314	0.0045	0.000024
Average	0.149	0.0207	0.000382	0.0082	0.000104
Death Valley	0.181	—	—	0.0305	0.000883
Mexico and Guatemala	0.357	—	—	—	—

If it be assumed that v and s are constant, $\sigma_{q(d)}^2 = 0$ by definition. With this assumption, equation (3) reduces to the following, giving $n = 285$.

$$n = 1/p_\infty. \tag{10}$$

Substitution of this value in (9) gives $v = 1.077 \times 10^{-5}$, and substitution in table 15 gives the values of $\bar{q}$, and of $\sigma_{q(c)}$ for stations and localities, respectively, in table 16.

In a random breeding population, the rate of change of the frequency of *chromosomes* carrying one or more completely recessive lethals was given as follows.

$$\Delta Q = V(1 - Q) - \frac{PQ^2(1 - Q)}{1 - PQ^2}. \tag{11}$$

The frequency of lethal bearing chromosomes should fluctuate only slightly about the value of Q at which $\Delta Q = 0$, which is approximately $\sqrt{V/P}$. The actual value of Q is in all cases much smaller, which requires

TABLE 16

Estimates of $\bar{q}$, of $\sigma_{q(e)}$, and $(\bar{s}+F)$ for station and locality, and of the last also for region, all on the assumption that $\sigma_{q(d)}^2=0$.

	$\bar{q}$	STATION $\sigma_{q(e)}$	LOCALITY $\sigma_{q(e)}$	STATION $(\bar{s}+F)$	LOCALITY $(\bar{s}+F)$	REGION $(\bar{s}+F)$
San Jacinto						
Keen	0.000554	0.00134	0.00058	0.0158	0.0183	(0.0189)
Pinon	0.000457	0.00094	0.00074	0.0213	0.0220	(0.0230)
Andreas	0.000546	0.00105	0.00029	0.0173	0.0190	(0.0192)
Average	0.000523	0.00116	0.00061	0.0177	0.0194	0.0201
Death Valley	0.000635	—	0.00176	—	0.0116	0.0163
Mexico and Guatemala	0.001252	—	—	—	—	0.0074

that lethals be eliminated more rapidly than under the conditions specified (random mating, elimination only in homozygotes). An increased rate of elimination may be due either to consanguineous mating or to adverse selection in heterozygotes or both.

The rate of change in the frequency of lethal *genes* is as follows, omitting terms involving q^3 and $\bar{s}Fq$ (compare the more complete formula (13) in preceding paper):

$$\Delta q = \bar{v}(1-q) - m(q-\bar{q}) - q(\bar{s}+F) - q^2(1-3\bar{s}-2F). \qquad (12)$$

By putting $\int_0^1 \Delta q\phi(q)dq = 0$ to express the constancy of the mean frequency of lethal genes

$$(\bar{s}+F) = [\bar{v}(1-\bar{q}) - (\sigma_{q(e)}^2 + \bar{q}^2)]/[\bar{q} - k(\sigma_{q(e)}^2 + \bar{q}^2)] \qquad (13)$$

where $k=3$ if $F=0$ and $k=2$ if $\bar{s}=0$ and in general k would be between 2 and 3. Estimates are given in table 16. The estimates of $(\bar{s}+F)$ for regions refer to regions sufficiently large that the frequency of each lethal would be at equilibrium. This is estimated by putting $\sigma_{q(e)}^2$ equal to zero in (13). These estimates are affected only slightly by the assumption made for n.

Joint estimates of N and m have been obtained as in the preceding paper by constructing the frequency distribution $f(q)(=\phi(q)/2N)$ for given $\bar{s}+F$, $\bar{v}$, n, and trial values of N and m.

$$f(q) = C\{1-2(\bar{s}+F)q-[1-(k-1)(\bar{s}+F)]q^2\}^{2N}q^{4N[m\bar{q}+\bar{v}]-1}(1-q)^{4Nm[1-\bar{q}]-1} \qquad (14)$$

It makes no appreciable difference what value of n is chosen. The minimum, $n=285$, was used. It also makes no appreciable difference whether k is taken as 2 or 3. An intermediate figure 2.5 was used. The frequency of the class of zero gene frequency was estimated from the formula

$$f(0) = f(1/2N)/4N(m\bar{q}+\bar{v}).$$

As an example, consider the case of the intra-station data from San

Jacinto. On substituting $n = 285$, $\bar{s}+F = 0.0177$, $\bar{v} = 0.00001077$, $m = 0.05$, $N = 2500$ and constructing ordinates at appropriate intervals beginning with 0, 0.0002, 0.0004, etc., the mean was found to be 0.00050 (instead of theoretical 0.00052) and the standard deviation was 0.00086 instead of theoretical 0.00116. Because this estimate of $\sigma_{q(c)}$ is too small, a smaller value of N was tried—namely, $N = 1000$—and ordinates were constructed at $q = 0$, $q = 0.0005$, $q = 0.0010$, etc. Calculation yielded $\bar{q} = 0.00051$ and $\sigma_{q(c)} = 0.00134$. This value of $\sigma_{q(c)}$ is too large. Hence the true value of N (for $m = 0.05$) must lie between 2500 and 1000. By interpolating between the logarithms of these values of N according to the differences in $\sigma_{q(c)}$, the estimate $N = 1400$ was arrived at. Calculations have been made by this method for the cases listed below, giving N to the nearest hundred (table 17).

TABLE 17

Estimates of effective size of population (N) for various values of the immigration coefficient (m) obtained by construction of the frequency distribution of q for trial values of N and m.

	m=0	m=0.01	m=0.02	m=0.05
Station				
Keen	3,400	—	—	1,100
Av. San Jacinto	4,100	2,900	2,200	1,400
Locality				
Keen	21,000	—	—	—
Av. San Jacinto	16,700	11,600	—	—
Death Valley 1937	2,400	—	—	900

The mean and standard deviation could be obtained directly if Δq were linear (*cf.* WRIGHT 1937). In the present case, Δq is not linear, but a rough approximation can be obtained by balancing the tendency toward increase in the frequency of the lethal—namely, $(\bar{v}+m\bar{q})(1-q)$ by the linear expression,

$$-\left(\frac{\bar{v}}{\bar{q}} + m\right)(1 - \bar{q})q$$

that gives the same mean, $\bar{q}$, as the correct expression.

If $\Delta q = -a(q-\bar{q})$, the deviation of gene frequency from the average changes in one generation from $(q-\bar{q})$ to $(1-a)(q-\bar{q})$. The mean sampling variance of $(q+\Delta q)$ is

$$\frac{1}{2N}\int_0^1 (q + \Delta q)(1 - q - \Delta q)\phi(q)dq = \frac{1}{2N}\left[\bar{q}(1 - \bar{q}) - (1 - a)^2\sigma_q^2\right]$$

At equilibrium

$$\sigma_{\bar{q}}^2 = (1-a)^2\sigma_q^2 + \frac{1}{2N}\left[\bar{q}(1-\bar{q}) - (1-a)^2\sigma_{\bar{q}}^2\right]$$

$$= \bar{q}(1-\bar{q})/\left\{2N\left[1-(1-a)^2\right] + (1-a)^2\right\}.$$

This includes the immediate sampling variance. But $\sigma_{q(\infty)}^2$ as determined by formula (4) is zero if $p = p_\infty$ and thus cannot include the immediate sampling variance. The above formula must accordingly be multiplied by $(1-a)^2$. In the present case $a = (m+\bar{v}/\bar{q})$ (to the degree of approximation used here). Solving for N:

$$N = \left[\frac{\bar{q}(1-\bar{q})}{\sigma_{q(\infty)}^2} - 1\right]\left(1 - m - \frac{\bar{v}}{\bar{q}}\right)^2 \Big/ 2\left[1 - \left(1 - m - \frac{\bar{v}}{\bar{q}}\right)^2\right] \quad (15)$$

The results agree sufficiently well for the present purposes with those obtained by trial and error from the formula for the distribution of gene frequencies. Some of the results are shown as the lower limits in table 19 and figure 2.

We must now consider the effect of variability in the values of v and s of different lethals.

Extreme heterogeneity with respect to the values of v does not appear to require much consideration. There is no indication in the present case that there are any loci that are giving rise to lethals with exceptionally high frequencies. There may be loci that give lethal mutations with extreme rarity, but if so, they would contribute little to either the mutations observed in the laboratory or found in nature. These can be ignored with restriction of n to loci that give lethal mutations with sufficient frequency to be of importance in the observations.

The greater chance of allelism within than between localities of San Jacinto (0.88% *vs.* 0.57%) might be due to differential selection, related to the marked ecological differences between the localities, instead of to limitation of numbers. This would not, however, account satisfactorily for the much greater chance of allelism within than between stations of the same locality at San Jacinto (2.13% *vs.* 0.88%) or within than between localities at Death Valley (3.11% *vs.* 0.41%) in which cases ecological differences are less pronounced.

The effect of extreme heterogeneity with respect to s (F being assumed to be zero at stations) was examined in the previous paper by postulating two classes of mutations, ones that are completely recessive and ones that are subject to very strong adverse selection as heterozygotes. It is possible for some of the mutations that occurred in the laboratory to be of the latter class, while most of those accumulating in natural populations are of the former class.

There is a third hypothetical class, lethal mutations that are subject to

favorable selection as heterozygotes and which therefore tend to reach exceptionally high frequencies in nature. Such lethals should be detected easily. Since there has been no indication of the occurrence of particular lethals at high frequencies, this possibility will be ignored.

As in the previous paper, we shall designate by subscript 1 the constants relating to completely recessive lethals—namely, n_1, $\bar{s}_1$ (=0), $\bar{q}_1$, etc., and by subscript 2 those relating to the class with strong adverse selection in the heterozygotes—namely, n_2, $\bar{s}_2$, $\bar{q}_2$, etc. There seems no reason for postulating different mean mutation rates for these classes. As in the previous paper, $\bar{q}_2$ is taken equal to v to give the most extreme possible case and $\bar{q}_2^2$ and $\sigma_{q(e)_2}^2$ are assumed to be negligibly small, permitting use of the following equations (*cf* p. 46, DOBZHANSKY and WRIGHT 1941).

$$n_1 v = p(n\bar{q})^2 \qquad (16)$$

$$n_2 v = V - n_1 v \qquad (17)$$

$$n_1 \bar{q}_1 = n\bar{q} - n_2 v \qquad (18)$$

$$n_1 \bar{q}_1^2 = p_\infty (n\bar{q})^2 \qquad (19)$$

$$n_1 \sigma_{q(e)_1}^2 = n_1 v - n\bar{q}_1^2 \qquad (20)$$

TABLE 18

Estimates based on the assumption that there are two classes of lethals, n_1 for which $\bar{s}+F=0$ within stations, and n_2 for which s is very high.

	n_1	n_2	v	$\bar{q}_1$	$\sigma_{q(e)_1}$ STATION	$\sigma_{q(e)_1}$ LOCALITY
San Jacinto						
Keen	276	1148	2.16×10^{-4}	0.000563	0.00136	0.00059
Pinon	273	2401	1.15×10^{-4}	0.000467	0.00096	0.00076
Andreas	275	1842	1.45×10^{-4}	0.000555	0.00107	—
Average	275	1560	1.67×10^{-4}	0.000533	0.00118	0.00062
Death Valley	279	551	3.70×10^{-4}	0.000653	—	0.00181

In dealing with localities, it was assumed that n_1, n_2, v, and $\bar{q}_1$ were the same as for stations, which implies that F is not zero in this case but is approximately equal to the excess of $\bar{s}+F$ in localities over that at stations as found under the hypothesis of uniform $\bar{s}+F$.

The results from the different localities are not consistent, especially with respect to n_2 and v. It is certain, however, that the hypotheses that $\bar{s}+F$ is zero for stations and that $\bar{q}_2=v$ are too extreme. The analysis is carried through merely to obtain upper limits for the values of N associated with given values of m_1.

In the case of San Jacinto stations, N was estimated by constructing frequency distributions with various trial values, assuming m=0. This gave N=30,000. Otherwise estimates were obtained by using formula (15). It may be noted that in the case referred to above this gave N=30,200. Some of the results are shown in table 19 and figure 2 (upper limit).

TABLE 19

Rough lower and upper estimates of effective size of population (N) on various assumptions with respect to immigration coefficient (m).

	m=0		m=0.01		m=0.05		m=0.20	
Stations	lower	upper	lower	upper	lower	upper	lower	upper
Keen	3,900–	20,000	2,500–	5,400	1,000–1,300		240–	260
Pinon	5,200–	52,000	3,600–	9,800	1,500–2,200		390–	430
Andreas	6,100–	46,000	4,000–	9,400	1,600–2,100		380–	420
Av. San Jacinto	3,600–	30,200	3,000–	7,100	1,200–1,700		300–	330
Localities								
Keen	20,200–	104,000	13,200–	28,000	5,500–6,800		1,300–	1,380
Pinon	8,400–	82,000	5,800–	16,000	2,500–3,500		630–	710
Av. San Jacinto	16,800–	111,000	11,100–	26,000	4,500–6,100		1,100–	1,220
Death Valley	2,900–	8,700	1,800–	3,100	680–	820	160–	170

These figures depend largely on the difference between the observed values of P and P_∞. This difference exceeds twice the standard error in the case of stations, in the average of the San Jacinto localities and in the Death Valley localities but is somewhat less than this in the data from the localities Keen and Pinon and is wholly without significance in the case of Andreas.

It may be seen from table 19 and figure 2 that the estimated upper limit is as much as tenfold greater than the lower limit in some cases, if m is assumed to be zero. The ratio is much less for m = 0.01 and becomes unimportant, considering other sources of uncertainty for values of m above 0.05.

In interpreting these figures we may note the following very rough estimates of the areas sampled in each case.

	STATION (square meters)	LOCALITY (square meters)	RATIO
San Jacinto			
Keen	10,000	6,000,000	600
Pinon	10,000	300,000	30
Andreas	5,000	50,000	10
Death Valley	—	200,000	—

It may be assumed that m is less than 0.01 in the locality Keen, indicating a value of N of the order 20,000 to 30,000 since close approach to the upper limit can safely be ruled out. The fact that this estimate is based largely on the percentage of allelism of lethals from flies of different generations (caught at intervals up to six months) would tend to make it too large, but only slightly, since the rate of change of gene frequency per generation is small compared with the standard deviation. The area sampled was about two square miles.

On this basis the population sampled at a typical station would have an effective size of only about fifty, but, as may be seen from figure 2, this implies such a high value of m (about 0.50) that the only effective isolation is that of a considerably larger area.

The estimates for station population at Pinon and Andreas are similar to those at Keen. This suggests that the effective density of population is

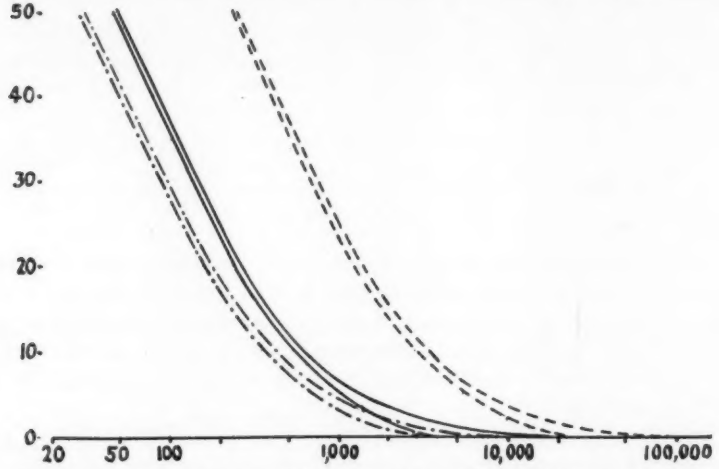


FIGURE 2.—The joint estimates of N, effective size of population (abscissas), and 100 m, replacement by immigration by representatives of the species (ordinates), indicated by the greater frequency of allelism of lethals from the same station or locality than of lethals from different localities. The solid lines are the upper and lower estimates for Keen stations. The broken lines are the corresponding estimates for the whole locality, Keen. The lines given by dash and dot are the estimates for Death Valley localities. These limits are those given by the extreme assumptions with respect to inbreeding and selection in heterozygotes and do not take account of the uncertainties of the figures due to small numbers.

about the same in all of these localities in spite of the differences in conditions. It is possible, however, that there are compensatory differences in N and m.

The value of m should be much larger for the locality Pinon than for the locality Keen because of the smaller area of the former. Accepting this, the estimation of N for Pinon comes out considerably smaller than for Keen and need not be more than about 30 times that of a station in Pinon as expected.

In Death Valley the effective size of population of localities appears at first sight to be smaller than at the San Jacinto stations in spite of their larger area. It is probable, however, that m is considerably smaller, permitting N to be a few times larger. Indeed, it may seem that m should be negligibly small in reference to the relatively much more isolated mountain

forests of Death Valley. It must be recalled, however, that each Death Valley collection was from only a small portion of a mountain forest. It is possible that the population of each of these forests was large enough (10^6 or more) for each lethal gene to be at approximately its equilibrium frequency in a sample representative of the whole forest. If this is the case, the isolation of the forests from each other would have no effect on the frequencies of the lethals. Thus, while m is probably considerably less than for a San Jacinto station, it should not be negligibly small. Another point that may be noted is that the Death Valley collections from a single locality were made at one time and therefore the estimates of N for given m applies more strictly to a single generation.

It is desirable at this point to consider the interpretation of the statistic $\bar{s}+F$. The upper limits of N considered above were on the hypothesis that F is zero (for stations) and that s is zero for most of the genes that accumulate in nature, but very high for most of the mutations observed in the laboratory, leading to their prompt elimination. This hypothesis was apposed by the absence of any evidence of such elimination in experiments in which mutations were allowed to accumulate and also by the similarity of the proportions of semilethals in nature and among the laboratory mutations. It led, moreover, to inconsistent estimates (of n_2 and v).

The hypothesis on which the lower limits of N were based—namely that s is constant (or nearly so) for all genes—led to certain estimates of $(\bar{s}+F)$. This was much smaller for Mexico and Guatemala (0.0074) than for San Jacinto (0.0201) or Death Valley (0.0163) (*cf.* table 16), reflecting the highly significant difference in lethal frequencies (table 4). This difference could be accounted for either by increased severity of selection against heterozygotes in the northern region or by more inbreeding. At first sight, the latter does not seem plausible since $(\bar{s}+F)$ for stations is as high as 0.0177 at San Jacinto, and it is obvious from the discussion above that there can be no isolation of subgroups within stations. It is possible, however, that there is enough tendency to brother-sister mating at times of the year when emerging broods are fewest to make a substantial contribution to F. The value of F for brother-sister mating is 0.25. The value of $(\bar{s}+F)$ for a typical San Jacinto station (0.0177) would require that about 7 percent of the matings be between brother and sister, if all other matings are random. It may be that there is no appreciable amount of brother-sister mating in the southern region where breeding is probably continuous throughout the year and that the value of $(\bar{s}+F)$ there measures wholly selection against heterozygotes. If the same selection applies in the northern region, it would require in addition about 4 percent of brother-sister matings to account for lower frequency of lethals. The data do not permit anything more definite than this survey of alternatives.

Although the lethal mutations have little direct bearing on the evolution of the species, the breeding structure of the population which is revealed by this study must be taken into account in considering possible mechanisms of evolutionary change. Unfortunately estimates of the immigration coefficient, m, of a given territory cannot be directly carried over from one character to another. The ideal case for analysis in terms of N and m is one in which a largely isolated colony with an effective population N receives migrants to the extent m, the migrants being genuinely representative of the species as a whole. In reality, a colony receives its migrants not from the entire species at random but mainly from adjacent localities. Populations of adjacent localities, however, tend to resemble each other to a greater extent than populations drawn from the entire distribution area of the species. For some genes the array of neighboring localities may show a gene frequency that approaches that in the species as a whole; for other genes the same array may be markedly different from the species at large. Thus, the mean frequency of each lethal gene should, as noted above, be close to its equilibrium frequency in a population with an effective size of the order 10^6 which, therefore, should be representative of the whole species. On the other hand, the population of *Drosophila pseudoobscura* is highly differentiated geographically with respect to the relative frequencies of various gene arrangements in the third chromosome (DOBZHANSKY and STURTEVANT 1938; also unpublished data). All the populations on Mount San Jacinto and in Death Valley region resemble each other with respect to these gene arrangements to a much greater extent than they do the populations of, for example, Texas or Mexico. The effective value of the immigration coefficient is therefore very much smaller for the gene arrangements than indicated for lethals.

SUMMARY

Samples of the population of race A of *Drosophila pseudoobscura* were obtained at three widely different localities on Mount San Jacinto in California. At one (Keen) the population minimum is in winter, at another (Pinon) there are two minima, winter and summer, at the third (Andreas) the minimum is in summer. Other data were obtained from a locality near Death Valley.

Among 1292 wild third chromosomes from flies caught in San Jacinto, 13.85 percent contained lethals and semilethals. This compares with 17.0 percent in 857 Death Valley chromosomes (including earlier data) and 30.0 percent in 120 chromosomes from Mexico and Guatemala. These exclude tests of a particular chromosome arrangement (Standard) in which the percentages were slightly less, probably because of technical difficulties.

There were no significant differences among the three "localities" on

San Jacinto or among the "stations" included in these localities. Collections were made throughout the year, but no significant differences were found in this respect.

These results contrast with the marked differences in the frequencies of different chromosome arrangements in different regions or among the three localities of San Jacinto (but not among the stations of the same locality).

The average percentage of allelism of lethals from populations so remote that common origin is largely excluded (different regions or localities) was 0.413 percent with a standard error of 0.081, including all available data. The percentage of allelism of lethals from different stations of the same locality was significantly higher (0.88 ± 0.20). It was still higher from flies from the same station (2.13 ± 0.32). It was somewhat higher in both cases for flies captured simultaneously than at intervals of one to six months apart, but the differences are not significant. These figures are somewhat lower than the figures previously published for flies from the same locality in Death Valley (3.11 ± 0.63).

An attempt was made to make deductions in regard to the breeding structure of the population and the properties of the lethal genes. The minimum number of loci capable of lethal mutation in the third chromosome is estimated at 285, corresponding to which is a mean mutation rate of 1.077×10^{-5} per locus per generation, a mean frequency per lethal of 0.00052 with a standard deviation of 0.00116 in "stations" and 0.00061 in "localities."

The frequency of lethals is much less than would be expected of completely recessive lethals subject to the observed mutation rate and with random mating. The data do not permit separation of the effects of inbreeding (coefficient F) and of selection against heterozygotes (coefficient $\bar{s}$) but indicate a joint estimate $(\bar{s}+F)=0.0177$ for stations, 0.0194 for localities, and 0.0201 for San Jacinto as a whole, the latter comparable to 0.0163 for Death Valley and 0.0074 for Mexico and Guatemala.

The higher percentage of allelism within stations and localities than at greater distances indicates that the effective size of population (N) in these areas is limited, but estimates can only be made contingent on assumptions with respect to the degree of isolation (immigration coefficient m). Effective N of the largest locality (Keen) area (about two square miles) is estimated at 2 or 3×10^4, assuming m much less than 1 percent. A typical station (diameter 100 yards) is estimated to have an effective N of only about fifty but with substantially no isolation from other stations in the locality.

LITERATURE CITED

DOBZHANSKY, TH., 1939 Genetics of natural populations. IV. Mexican and Guatemalan populations of *Drosophila pseudoobscura*. Genetics 24: 391–412.

DOBZHANSKY, TH., and M. L. QUEAL, 1938a Genetics of natural populations. I. Chromosome variation in populations of *Drosophila pseudoobscura* inhabiting isolated mountain ranges. Genetics **23**: 239–251.

DOBZHANSKY, TH., and M. L. QUEAL, 1938b Genetics of natural populations. II. Genic variation in populations of *Drosophila pseudoobscura* inhabiting isolated mountain ranges. Genetics **23**: 463–484.

DOBZHANSKY, TH., and A. N. STURTEVANT, 1938 Inversions in the chromosomes of *Drosophila pseudoobscura*. Genetics **23**: 28–64.

DOBZHANSKY, TH., and S. WRIGHT, 1941 Genetics of natural populations. V. Relations between mutation rate and accumulation of lethals in populations of *Drosophila pseudoobscura*. Genetics **26**: 23–51.

KOLLER, P. CH., 1939 Genetics of natural populations. III. Gene arrangements in populations of *Drosophila pseudoobscura* from contiguous localities. Genetics **24**: 22–33.

WRIGHT, S., 1937 The distribution of gene frequencies in populations. Proc. Nat. Acad. Sci. **23**: 307–320.

PREFERENTIAL SEGREGATION IN MAIZE

M. M. RHOADES
Columbia University, New York City

Received December 25, 1941

THE orientation of a bivalent on the metaphase plate is usually at random so that the distribution of any one of the two homologues to a specific pole of the spindle figure is wholly a matter of chance. The validity of this statement is established by the large number of cases in many diverse organisms in which 1:1 genetic ratios are obtained when the two homologous chromosomes are marked by contrasting alleles and when there are no complications due to viability differences of the two phenotypes. Preferential segregation (that is, the passing of a specific chromosome to a specific pole) may be detected genetically in oogenesis and megasporogenesis since normally only one of the four nuclei arising from the two meiotic divisions survives. Here preferential segregation will give rise to distorted genetic ratios. Since a number of mechanisms are known which produce distorted genetic ratios, all other possible causes must be shown to be non-operative before any aberrant ratio can be ascribed to preferential segregation. This is often a difficult task, and few cases of non-random segregation have been established.

Results obtained in the course of some investigations with an abnormal type of chromosome 10 in maize are apparently explicable only on the assumption of preferential segregation in megasporogenesis. This paper presents the data which justify such a conclusion.

EXPERIMENTAL RESULTS

LONGLEY (1937, 1938) found an unusual type of chromosome 10 in four strains of maize grown by Indian tribes in the southwestern part of the United States and also in a strain of teosinte from Chapingo, Mexico. This unusual type of chromosome 10 differs from the chromosome 10 found in all other tested strains of maize in having an extra piece of chromatin attached to the end of the long arm. The length of this piece is about that of the short arm of chromosome 10 (fig. C, D). The proximal portion of the attached piece is euchromatic, as is a smaller distal portion. A large and conspicuous knob comprising approximately one-half of the pachytene length lies between the two euchromatic portions. The relative length of the extra piece is much greater at late diakinesis than at pachytene, since at diakinesis it constitutes a bulk equal to the entire length of a normal chromosome 10. This is due to the fact that the large pycnotic knob undergoes a comparatively slight reduction in size as meiosis advances while the euchromatic portions become greatly contracted. Nothing is known of the

origin of this extra piece. It is not morphologically similar to the B chromosomes, and insofar as the present tests indicate, it carries no loci found in the normal chromosome 10. No pairing of the attached piece with any of the other chromosomes composing the regular complement has been observed.

A strain of maize homozygous for the abnormal chromosome 10 was obtained through the kindness of DR. LONGLEY. It was originally planned to use this abnormal type of determine the amount of recombination between the *R* locus and the distal end of the long arm of chromosome 10. It is known from the studies of MCCLINTOCK (1931), STADLER (1933), and ANDERSON (1935) that the *R* gene is in the distal portion of the long arm.

The abnormal chromosome carried the recessive *r* allele. Crosses were made between plants with the abnormal tenth and those with the dominant *R* allele from two unrelated normal strains. Both sets of F_1 plants were backcrossed to strains with normal chromosomes 10 possessing the *r* allele and homozygous for the dominant alleles of the other loci concerned with aleurone color. In these heterozygous plants the abnormal chromosome carried the *r* allele while the normal tenth bore the *R* allele, and a ratio of one colored to one colorless was expected in the backcross progenies. When the F_1 plants from one of the two original crosses were pollinated with *r* pollen, a total of 1881 *R r* and 4441 *r r* seeds (29.8 percent *R*) was obtained on 15 backcrossed ears instead of the expected 1:1 ratio. The second set of F_1 plants gave a total of 1092 *R r* and 3409 *r r* kernels (24.3 percent *R*) on ten ears similarly pollinated. Although the *R*:*r* ratio was far from the expected 1:1, it is evident that all *R r* plants should have two normal chromosomes 10 and that *r r* plants should have one abnormal and one normal chromosome 10 if there was no crossing over between *R* and the point of attachment of the extra piece. The constitution of 100 plants from *R r* and *r r* seeds was determined. Crossover types would be *r r* plants with two normal chromosomes and *R r* plants with one abnormal and one normal chromosome. One crossover was found in the 100 tested plants; in this case a plant of *R r* constitution had an abnormal chromosome with the *R* allele. The close linkage of the *R* locus and the extra piece indicated that the aberrant *R*:*r* ratio was due to a peculiar behavior of the abnormal chromosome resulting its being included in more than half of the functioning ovules. The close linkage of the *R* locus with the extra piece made this locus a valuable tool in following the two types of chromosomes 10 in subsequent breeding experiments.

Additional data on the amount of recombination between *R* and the end was obtained by purely genetic means. The *R*:*r* ratio was used to identify the two types of chromosome 10 in backcross progenies. Colored seeds produced by crossing a normal *r* strain with pollen of abnormal *r*/normal *R*

heterozygotes should carry two normal chromosomes if no crossing over occurred. Plants with non-crossover chromosomes should give 1:1 ratios of *R*:*r* when used as the female parent in backcrosses. Crossovers, with the *R* allele in the abnormal chromosome, would give a marked excess of colored seeds above a 1:1 ratio. (It has been concluded that preferential segregation is responsible for these distorted ratios. The evidence permitting such a conclusion will be presented later.) It is assumed that when a normal chromosome receives the extra piece through crossing over, this newly formed abnormal chromosome will undergo preferential segregation as did the original abnormal chromosome—that is, the extra piece is the cause of the non-random segregation. Data showing that this is true will be given below. A total of 633 gametes were tested by this method; 620 were non-crossovers while 13 or two percent were crossovers between *R* and the attached piece. In most of the ears there was no doubt of the correct classification, but in a few cases it was necessary to check the inferred constitution by cytological examination or further breeding tests. The low percentage of recombination found by this method is in agreement with that found by direct cytological examination as previously described.

Counts of colored and colorless seeds were made on 131 backcrossed ears from plants heterozygous for the two types of chromosome 10. The abnormal chromosome carried the *R* allele by virtue of an earlier crossover. A total of 27,470 *R r* (colored) and 10,515 *r r* (colorless) seeds, a percentage of 72.3 *R*, was found. Since the *R* locus is so closely linked to the attached piece, the ratio of colored to colorless kernels is approximately equal to the ratio of abnormal to normal chromosomes 10. It follows that about 72 percent of the ovules received the abnormal chromosome. There was a total of 4,806 *R r* and 10,939 *r r* seeds, a percentage of 69.5 *r*, on 43 backcrossed ears where the *R* allele was in the normal tenth and the *r* allele in the abnormal chromosome. These data indicate that approximately 70 percent of the ovules have received the abnormal chromosome. The two sets of crosses, differing in phase of linkage, agree very well on the degree of preferential segregation.

The above data indicate that it is the extra piece attached to the end of the long arm which is responsible for preferential segregation. When the *R* allele was shifted to an abnormal chromosome through crossing over, a considerable portion of the normal chromosome was transferred concomitantly, yet the newly formed abnormal chromosome underwent preferential segregation as before. That the extra chromatin is the effective agent producing non-random segregation was further demonstrated by replacing large portions of the original abnormal chromosome with chromatin known to be free of factors causing abnormal ratios. For example,

chromosomes with the *li g R* alleles, derived from a normal chromosome, and the extra piece undergo preferential segregation. The *li* locus is about 35 crossover units to the left of *R*. It is evident that the distal part of the long arm of chromosome 10 is free from genetic factors producing the unusual distribution of the abnormal chromosome.

Before accepting the correctness of the preferential segregation hypothesis, it was necessary to prove that other mechanisms were not at play. It seemed possible that equal numbers of ovules with the two types of chromosomes 10 were formed and the the deficiency of one type was due to abortion. For example, RHOADES and RHOADES (1939) have shown that the *sp2* gene, which is situated in chromosome 10, causes the abortion of 44 percent of the *sp2* ovules. A similar abortion-inducing gene might account for the present deviation from a 1:1 ratio. If this factor were located near the *R* locus it would produce a deficiency of ovules possessing this gene and consequently distort the *R*:*r* ratio. But the abortion of a number of ovules carrying the normal chromosome sufficient to give the observed deviation from equality would produce a noticeable sterility on the ear. No such sterility was found, as figure A illustrates. The fertile spikelets of the maize cob are paired—a condition which makes possible the detection of even a slight degree of abortion if the ears are carefully examined. The maximum sterility on three closely inspected ears with marked deviations in the *R*:*r* ratio was six percent, a value far too small to account for the deviation from a 1:1 ratio. Furthermore, the normal tenth chromosomes used in these experiments are free from ovule abortion genes. Extensive control data on the *R*:*r* ratio from plants with normal chromosomes 10 gave an extremely good fit to a 1:1 ratio. Four hundred and four ears from the backcrossing of heterozygous plants had a total of 53,740 *R r* (colored) and 53,492 *r r* (colorless) seeds. The absence of sterility on the ear and the freedom of the normal chromosome 10 from ovule abortion genes negate the abortion hypothesis. Normal 1:1 ratios of *R*:*r* in both male and female flowers were obtained from plants homozygous for the abnormal chromosome.

The above data indicate that the two types of chromosome 10 were never represented with equal frequencies in the ovules of heterozygous plants. Such a condition could arise from megaspore competition. The basal (chalazal) one of the linear series of four megaspores of maize gives rise to the embryo sac, while the other three megaspores degenerate. The distorted ratios reported in these experiments could arise if non-basal megaspores with the abnormal chromosome frequently competed successfully with the basal megaspores containing the normal chromosome 10 and formed the embryo sac. Such a phenomenon has been reported by RENNER (1921) for *Oenothera muricata*, where megaspores with the *rigens* complex

develop into the functioning embryo sac irrespective of their position in the row of four megaspores. In order to determine if megaspore competition is responsible for the distorted ratios observed in the present experiments, the development of more than 200 embryo sacs was followed in plants heterozygous for the abnormal chromosome. Here, as normally, the basal megaspore always developed into the embryo sac, while the other three megaspores degenerated. Megaspore competition, then, does not account for the unusual breeding results reported in this paper.

The preferential segregation hypothesis was accepted only after the elimination of other explanations. Although it was not verified by cytological observations of anaphase disjunction during megasporogenesis because of technical difficulties (the aceto-carmine smear technic is not feasible for this tissue, and sectioned material is not satisfactory), it satisfactorily accounts for all known facts. It is believed that the orientation of the heteromorphic bivalent on the spindle figure is non-random so that the abnormal chromosome passes to the lower pole of the spindle in a majority of the cases. The genetic length of the long arm of chromosome 10 is such that an average of at least one chiasma is formed between the centromere and the distal end. If one chiasma always occurred, anaphase I would be equational for the extra piece, and the dyads in both of the telophase nuclei would consist of one normal and one abnormal chromatid. Preferential segregation then would occur at the second division, with the abnormal chromosome passing to the lower pole, from which the basal megaspore arises. It may be that preferential segregation invariably occurs at anaphase II when a heteromorphic dyad is present. If such be the case, then the ovules with normal chromosomes 10 would come from those anaphase I disjunctions where two normal chromatids pass to the basal pole. It is doubtful if preferential segregation is obligatory at anaphase II when heteromorphic dyads are present; it seems more probable that the abnormal chromatid has a propensity to go to the basal pole and that this inclination is subject to modification.

TRANSMISSION OF THE ABNORMAL CHROMOSOME THROUGH POLLEN

We have seen that more than 70 percent of the ovules of heterozygous plants receive the abnormal chromosome, and this preponderance has been ascribed to preferential segregation. Since the basal megaspore always gives rise to the embryo sac, there is no competition between megaspores with normal and those with abnormal chromosomes. However, in the development of the male gametes all four cells of each quartet formed by the two meiotic divisions develop into pollen grains. Two of the four have the normal and two the abnormal chromosome. Pollen tube growth is highly competitive, and any difference in pollen tube growth between the

two kinds of pollen would give distorted *R*:*r* ratios. Forty-nine pairs of direct and reciprocal crosses were made between individuals of normal *r* strains and those heterozygous for the abnormal chromosome. When heterozygous plants were used as the female parent, there was a total of 10,542 *R r* (colored) and 5,064 *r r* (colorless) seeds on the 49 ears (68 percent *R*). The reciprocal cross, in which the heterozygous plants were used as the pollen parent, had a total of 6,705 colored and 9,225 colorless seeds (42 percent *R*). This percentage is significantly less than the expected 50 percent. The *R* locus is so close to the point of attachment of the extra piece that the ratio of *R*:*r* is a good approximation of the relative frequencies of the abnormal and normal types of chromosome 10. These data indicate that the extra piece has a deleterious effect on the growth of the pollen tube. Data comparable to the above were obtained from heterozygous plants with the abnormal chromosome carrying the *r* allele. On a total of 31 ears there were 3,801 colored and 3,470 colorless kernels. The deficiency of functioning grains with the abnormal chromosome is less here than in the first experiment, but the deviation from equality is highly significant. The deviation through the male gametes is the reverse of that through the female flowers.

The conclusion that there is unequal transmission of the two types of chromosome 10 through the pollen is contingent upon the two chromosomes being represented equally among the pollen grains and also upon 1:1 ratios being obtained with two normal chromosomes 10. While an unusual situation was encountered in certain plants involving the formation of spindle fibers by regions of the chromosome other than the centromere, this was a phenomenon affecting all the chromosomes, and there was no indication that the abnormal chromosome was lost through failure of disjunction during microsporogenesis. There was no more than the usual amount of pollen sterility in the heterozygous plants, so the evidence, while not conclusive, lends no support to the suggestion that the two types of chromosome 10 might be unequally represented in the pollen sample. The control data through the pollen are as follows: There were 4,428 *R r* and 4,333 *r r* kernels (50.5 percent *R*) on 40 backcrossed ears using plants with normal chromosomes 10 heterozygous for *R* as the pollen parent. The deviation from 50 percent is not significant. The lowered transmission through the pollen of the abnormal chromosome was possibly due to the action of active loci carried in the euchromatic portions of the extra piece resulting in hyperploidy. It is well known that in maize hyperploid grains usually do not successfully compete against haploid grains. While the duplication hypothesis has been advanced to account for the decreased transmission of abnormal chromosomes through the male gametes, nothing is known of the nature or origin of the chromatin composing the extra piece.

It is conceivable that the abnormal chromosome is deficient for the tip of chromosome 10 even though it carries extra chromatin of uncertain origin. Such a duplication-deficiency chromosome could arise from a translocation followed by aneuploid segregation. If the abnormal chromosome possesses, in addition to the extra chromatin, all loci normally present in chromosome 10, then it is probable that the extra piece has been inserted into the long arm near the distal end and is not terminally attached, since simple translocations are not known to occur. However, a study of pachytene pairing in heterozygous cells shows in most cases a close synapsis of the two chromosomes up to the distal end of the normal chromosome, suggesting a terminal attachment of the extra piece. The occurrence of non-homologous pairing in maize makes such a conclusion hazardous. LONGLEY (1937) suggested that the abnormal chromosome might be the ancestral form and that the type of chromosome 10 now found in nearly all strains arose as a deletion. If this is so, then the extra piece is truly terminal. While it is not possible to disprove this postulated origin, there is no evidence favoring it, and several factors weigh against it.

EFFECT OF THE EXTRA CHROMATIN ON CROSSING OVER

There is one or two percent of crossing over between *R* and the point of attachment of the extra piece. The observed recombination value is probably less than the map distance between *R* and the tip of the normal chromosome. ANDERSON (unpublished) finds about four or five percent recombination between *R* and a distally placed break in a translocation heterozygote, and there is an unknown amount of crossing over in the small terminal portion of chromosome 10 moved to the other chromosome. If the extra piece of the abnormal chromosome is not terminally attached but is inserted near the end of the long arm, then the low percentage of recombination distal to *R* found in these experiments is probably due to the interference of the inserted piece with synapsis of that part of the chromosome distal to the inserted piece. The close pairing observed at pachytene suggests that asynapsis is not the cause of the low crossover value. (If the insertion hypothesis is correct, the tip of the normal chromosome is non-homologously paired with the proximal euchromatic part of the extra piece.) The intimate synapsis observed here is in contrast to the frequent asynapsis found when a heterozygous terminal knob is present. The difference may be due to the fact that the portion of the extra piece adjacent to the end of the long arm is euchromatic rather than heterochromatic. However, any reduction, whatever the cause, is confined to the region distal to *R*, as is shown by the following data. The golden (*g*) locus lies 14 units to the left of *R*. Plants of *G r* abnormal/*g R* normal constitution were used as the male and female parents in backcross tests. The more extensive data on crossing over in the female flowers are given in table 1.

These data show that the amount of crossing over between *g* and *R* (14 percent) is the same as occurs in stocks with normal chromosomes 10. The data in table 1 also show that the distortion of the *G*:*g* ratio from the usual 1:1 is less than that of the *R*:*r* ratio. This, of course, is due to the fact that the golden locus is further removed in crossover units from the extra piece than is the *R* locus. A gene located in the short arm of chromosome 10 would be expected to be little affected by the extra piece. The data in table 1 further show that crossing over in the *g R* interval has no effect on preferential segregation, since the ratio of *R* to *r* is approximately the same in both crossover and non-crossover classes. The amount of recombination between *g* and *R* was 17 percent in the male flowers, but the population

TABLE 1

Linkage data from the cross of G r abnormal/g R normal×g r.

LINKAGE PHASE	CONSTITUTION OF CHROMOSOMES					RATIO OF *R*:*r* ON EAR
Repulsion	(o) *G* *r*	(o) *g* *R*	(X) *G* *R*	(X) *g* *r*	Total	
	243	138	29	49	459	186*R* : 326*r*
	102	86	9	13	210	136*R* : 319*r*
	150	114	18	20	302	145*R* : 288*r*
	396	50	7	59	512	169*R* : 588*r*
	154	81	11	29	275	120*R* : 277*r*
	1045	469	74	170	1758	756*R* : 1798*r*

consisted of only 345 individuals, and the difference in crossing over between the male and female flowers is not significant. RHOADES and RHOADES (1939) found no differences in crossing over in several regions of chromosome 10 in the male and female flowers.

EFFECT OF THE ENVIRONMENT ON PREFERENTIAL SEGREGATION

In the summer of 1939 F_1 plants from the cross of the original abnormal strain by a normal *R* strain were pollinated with *r* pollen. As reported earlier, ten backcrossed ears (family 6471) had 1,092 colored and 3,409 colorless kernels, percentages of 24.3 *R* and 75.7 *r*. Remnant seed of the same F_1 ear were planted in 1940 as family 7233 and were similarly backcrossed. Eighteen ears had 1,833 *R r* (colored) and 3,089 *r r* (colorless) kernels, percentages of 37.2 *R* and 62.8 *r*. The marked difference in the percentages of *R* and *r* kernels for the two seasons is highly significant. The two lots of seed had the same average genetic constitution. The significant difference in the degree of preferential segregation for the two seasons has been ascribed to the influence of environmental factors, the nature of

which can be only surmised at present. Perhaps a marked difference in temperature existed at the critical period (presumably the meiotic divisions). This supposition gains some support from the fact that SEILER (1921) found the behavior of the X chromosome in *Talaeporia tubulosa* to be markedly influenced by the temperature level. He reported that with normal temperatures, and also with subnormal temperatures, the X most frequently passed into the polar body, but under high temperatures, and also in overripe eggs, the X was more often left in the egg.

If the degree of preferential segregation is affected by inter-seasonal environmental differences, it might be expected that intra-seasonal variation would also affect preferential segregation. If such be the case, then the frequency of eggs with the abnormal chromosome would vary considerably among the different plants of a family, since the meiotic divisions would not occur simultaneously in all the plants. There would be ample opportunity for environmental changes to take place, especially temperature differences. If a varying environment affects preferential segregation, it would result in heterogeneity of the percentages of colored and colorless seeds on sister ears. The degree of preferential segregation, as reflected in the ratio of colored and colorless seeds, was determined for 12 groups (families) of sister plants. The χ^2 test for homogeneity was made within each of these groups. In only one group consisting of ten ears, family 6471, did the percentages of *R* and *r* kernels on the sister ears suggest that they formed a homogeneous population. A marked heterogeneity was found within the ears comprising family 7233. The analysis of variance of the data in families 6471 and 7233 showed that the variance between seasons was significantly greater than that within seasons, since the mean square between seasons was more than 11 times that within seasons. This indicates that greater environmental differences existed at the time of meiosis between the two seasons than within. The heterogeneity among sister ears grown the same season and the significant difference in variance between seasons can both be ascribed to changes in the environment. This is true only if the marked deviations of individual ears from the mean of the population are not due to genetic differences. A percentage of 72.3 *R* seeds was obtained on 131 ears when the *R* allele was in the abnormal chromosome. A few ears had percentages of colored and colorless seeds differing significantly from the mean. Such differences might be due to genetic modifiers, but data were obtained showing that these ears with significant deviations from the mean do not transmit this condition to the next generation. For example, ear 7229-14 had 137 *R r* and 138 *r r* kernels, a significant deviation from the expected 199:76 (calculated by using the mean percentage of 72.3 *R*) Cytological examination proved that the abnormal chromosome was heterozygous; hence the 1:1 ratio was not

due to a crossover having occurred in the previous generation transferring the *R* allele to a normal chromosome 10. Colored seed from ear 7229–14 was planted and the ensuing plants backcrossed with *r* pollen. There were 7,215 *R r* (73.3 percent) and 2,630 *r r* seeds on 27 ears. It may reasonably be concluded that the lack of preferential segregation in ear 7229-14 cannot be laid to a genetic agent but may be attributed to some external influence affecting the mechanics of chromosome movement.

RELATION OF PREFERENTIAL SEGREGATION TO FORCE OF GRAVITY

It has been concluded that the abnormal chromosome passes to the lower pole of the spindle during megasporogenesis. The length of the extra piece at late diakinesis is approximately equal to that of the normal chromosome 10. The longitudinal axes of the meiotic spindles are approximately parallel to the longitudinal axis of the main stalk, which normally grows vertically. The equatorial plate of the meiotic spindle is at right angle to the force of gravity. If, by virtue of possessing the large and presumably heavy pycnotic knob in the extra piece, the tendency of the abnormal chromosome to pass to the lower pole was a response to the power of gravity, then inverting the plants 180 degrees at the time of meiosis should result in the abnormal chromosome now passing as before to the lower pole, with reference to gravity, but to one which gives rise to a non-basal megaspore. Normal chromosomes 10 should be found in more than half of the basal megaspores. Heterozygous plants which had been growing in an upright position were inverted prior to the onset of meiosis and left in this position until the silks had extruded and were ready for pollinating. A number of sister plants were left in an upright position. Both sets of plants were backcrossed by recessive *r* pollen. Eight control plants had a total of 959 *R r* and 314 *r r* kernels (75.3 percent *R*), while seven inverted plants had a total of 599 *R r* and 209 *r r* kernels (74.1 percent *R*). Apparently the preferential disjunction of the abnormal chromosome to the lower pole is not a response to the force of gravity.

DISCUSSION

The conclusion has been reached that preferential segregation is the cause of the high frequency with which the abnormal chromosome is recovered among the ovules of heterozygous ears. It has been demonstrated that ovule abortion and megaspore competition are not the causal agents of the distorted ratios. The results here presented are unique, although cases of determinate disjunction or preferential segregation are known. For example, SCHRADER (1931) observed in Protortonia that the five chromosomes in the secondary spermatocytes were not oriented on the equatorial plate in the usual fashion but were arranged in a straight line

between the two poles, with two chromosomes going to one pole and three to the other. Evidently some sort of regulation is involved so that the alignment of the five chromosomes is not at random, else a definite complement of chromosomes would not pass to the two poles. METZ (1938) reported that monocentric mitoses occurred in Sciara, with the paternal set of chromosomes passing away from the pole and finally degenerating. The chromosomes derived from the mother go to the functional pole and hence are transmitted to the next generation. DOBZHANSKY (1935) presented genetic evidence that the unpaired X^2 chromosome of the *Drosophila miranda* male passes to the same pole as the X^1 chromosome, which regularly disjoins from the Y with which it forms a bivalent. BRIDGES, in MORGAN, BRIDGES, and STURTEVANT (1925) found that in triploids the distribution of the chromosomes is not according to chance. An interdependence of such a nature exists whereby certain combinations of sex chromosomes and autosomes are formed more often than others.

STURTEVANT (1936) described an extensive series of crosses involving segregation in triplo-IV females of *Drosophila melanogaster*. He found that the segregation of the three chromosomes IV was not at random but that certain of the chromosomes passed to the same pole more frequently than expected with random segregation. MATHER (1939) mentioned that in sc^8/sc^8-def. females of *Drosophila melanogaster* the sc^8-def. chromosome was recovered twice as frequently among the daughters as was the sc^8 chromosome. MATHER was concerned with other considerations and did not determine the cause of the deviating ratio but stated that it probably was a maternal effect. It may be that non-random disjunction was responsible. The work of SEILER has already been cited.

It is clear, however, that with the possible exception of MATHER'S case, none of the above examples is similar to the present account. We are here concerned with the preferential segregation of one member of a heteromorphic pair. The above examples involve non-random segregation of sets of chromosomes, of individual members of a trivalent group, or of unpaired chromosomes. But all of these cases including the present one share a common feature in that they disclose unsuspected forces affecting chromosome movement and segregation.

SUMMARY

An abnormal type of chromosome 10, found by LONGLEY in maize from the southwestern part of the United States, is preferentially segregated during megasporogenesis. More than 70 percent of the ovules receive the abnormal chromosome instead of the 50 percent expected with random segregation. At pachytene the length of the extra piece of chromatin in the abnormal chromosome is slightly greater than the short arm of chromo-

some 10. The proximal and distal portions of the extra piece are euchromatic, but a large and conspicuous knob lies between the two euchromatic portions. The origin of this extra piece is unknown.

Pollen with the abnormal chromosome 10 is only partially successful in competing with pollen possessing a normal chromosome 10. It is suggested that extra chromatin present in the abnormal chromosome produces a deleterious effect on pollen tube growth.

The excess of ovules with the abnormal type of chromosome is due neither to abortion of ovules with a normal chromosome nor to megaspore competition.

The *R* locus, known to lie in the long arm of chromosome 10, proved to be closely linked to the extra piece of chromatin which is believed to be inserted near the tip of the long arm. The observed percentage of recombination (one or two percent) is probably less than the amount occurring distal to *R* in stocks carrying two normal chromosomes 10. Crossing over in the *g R* region is not affected when the abnormal chromosome is heterozygous, and crossing over in this interval did not affect preferential segregation.

Evidence was obtained indicating the influence of the environment on the degree of preferential segregation.

ACKNOWLEDGMENTS

The writer wishes to express his gratitude to VIRGINIA H. RHOADES who made most of the earlier cytological observations, and also to HILDA VILKOMERSON for technical assistance.

LITERATURE CITED

ANDERSON, E. G., 1935 Chromosomal interchanges in maize. Genetics **20**: 70–83.

DOBZHANSKY, TH., 1935 *Drosophila miranda*, a new species. Genetics **20**: 377–391.

LONGLEY, A. E., 1937 Morphological characters of teosinte chromosomes. J. Agric. Res. **54**: 835–862.

1938 Chromosomes of maize from North American Indians. J. Agric. Res. **56**: 177–195.

MATHER, K., 1939 Crossing over and heterochromatin in the X chromosome of *Drosophila melanogaster*. Genetics **24**: 413–435.

MCCLINTOCK, B., 1931 Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Missouri Agric. Expt. Sta. Research Bull. **163**: 1–30.

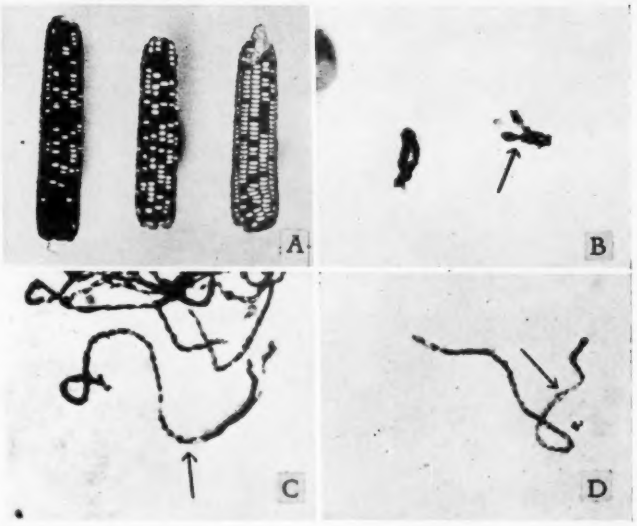
METZ, C. W., 1938 Chromosome behavior, inheritance and sex determination in Sciara. Amer. Nat. **72**: 485–520.

MORGAN, T. H., C. B. BRIDGES, and A. H. STURTEVANT, 1925 The genetics of Drosophila. Bibliogr. Genet. **2**: 1–262.

RENNER, O., 1921 Heterogamie im weiblichen Geschlecht und im Embryosackentwicklung bei den Oenothera. Z. f. Bot. **13**: 609–621.

RHOADES, M. M., and V. H. RHOADES, 1939 Genetic studies with factors in the tenth chromosome in maize. Genetics **24**: 302–314.

SCHRADER, F., 1931 The chromosome cycle in *Protortonia primitiva* (Coccidae) and a consideration of the meiotic division apparatus in the male. Z. wiss. Zool. **138**: 386–408.



EXPLANATION OF FIGURES A–D

FIGURE A.—The ear on the left is from the cross of *R* abnormal */r* normal times *r r* pollen and illustrates the marked excess of *R* (colored) seed over a 1:1 ratio. The abnormal chromosome bearing the *R* allele is preferentially segregated to the basal pole during megasporogenesis. A backcrossed ear from a plant carrying two normal chromosomes is shown in the middle. Here the expected 1:1 ratio of colored to colorless seed is apparent. The ear on the right is from the cross of *r* abnormal */R* normal times *r r* pollen and again illustrates the striking excess of ovules receiving the abnormal chromosome. Note that there is no indication of sufficient sterility on the ear to account for the deficient class.

FIGURE B.—Diakinesis of bivalent composed of two abnormal chromosomes 10. The large pycnotic knob is clearly visible at this stage. The arrow indicates the approximate position of the proximal end of the extra piece of chromatin. At late diakinesis the knob portion of the extra piece seems to be equal in size to a normal chromosome 10. This is best seen in heterozygous material. There are no chiasmata in the extra piece in this figure, and none is usually found.

FIGURE C.—Pachytene of homozygous abnormal chromosomes 10. The arrow marks the approximate position of the proximal end of the extra piece. In this figure the pycnotic (knob) region is longer and slenderer than in many cells, while the distal euchromatic portion is shorter and more deeply stained than usual.

FIGURE D.—Pachytene of heterozygous bivalent. The arrow indicates the proximal end of the extra piece. The distal euchromatic portion of the extra piece is not visible in this preparation because it has non-homologously paired with the pycnotic portion. This frequently occurs.

SEILER, J., 1921 Geschlechtschromosomenuntersuchungen an Psychiden. I. Experimentelle Beeinflussung der geschlechtsbestimmenden Reifeteilung bei *Talaeporia tubulosa* Retz. Arch. Zellforsch. **15**: 249–268.

STADLER, L. J., 1933 On the genetic nature of induced mutations in plants. II. A haplo-viable deficiency in maize. Missouri Agric. Expt. Sta. Research Bull. **204**: 1–29.

STURTEVANT, A. H., 1936 Preferential segregation in triplo-IV females of *Drosophila melanogaster*. Genetics **21**: 444–466.

AN EMBRYOLOGICAL AND GENETICAL STUDY OF RANA BURNSI WEED[1]

JOHN A. MOORE
Queens College, Flushing, N. Y.

Received January 20, 1942

IN NATURAL populations of meadow frogs from the north central states an occasional individual is found that differs markedly in pigmentation from the typical *Rana pipiens*. The latter has the back, sides, and legs covered with black spots of various sizes and shapes. The exceptional individuals closely resemble *pipiens* in general form but are completely or nearly devoid of the black spots (fig. 1, 2). These "meadow frogs without spots" have been treated in a variety of ways by taxonomists. DICKERSON (1906) regarded them as a color variety of *pipiens* and was apparently of the opinion that the condition occurred only in juvenile frogs. In 1922 WEED suggested they be regarded as a distinct species and given the name *Rana burnsi*. This step was deemed necessary, since both spotted and non-spotted individuals are found together but with no evidence of intergradation. WEED'S species has not been universally accepted. Neither KELLOGG (1932) nor STEJNEGER and BARBOUR (1939) recognize *Rana burnsi*. WRIGHT and WRIGHT (1933) have provisionally listed it as a subspecies of *pipiens*. Academic treatment of this kind, however, can do little to settle the problem.

A few years ago a shipment of *Rana pipiens* arrived from the middle west which included several *Rana burnsi*. This provided an opportunity for studying the early development of this non-spotted variety and for attempting to secure hybrids with typical *Rana pipiens*. Cross fertilization between *burnsi* and *pipiens* was successful, and a few tadpoles were kept until metamorphosis. In the controls, *burnsi* ♀ × *burnsi* ♂, three tadpoles transformed. These were of two kinds—one non-spotted like the parents, the other two spotted and indistinguishable from ordinary *pipiens*. The hybrids of *pipiens* and *burnsi* similarly gave rise to two types of offspring. Of the 24 transforming in this cross, 13 were non-spotted and 11 spotted. There was no evidence of blended inheritance. Seven controls, *pipiens* ♀ × *pipiens* ♂, differed in no way from their parents.

These rather surprising results suggested that the non-spotted frogs that WEED had named *Rana burnsi* differed from the typical *Rana pipiens* by one dominant gene carried in the heterozygous state. Experiments with *Rana burnsi* were resumed the following year. The problem was assailed

[1] These experiments were performed in the Department of Zoology, Columbia University, and in the Department of Biology, Brooklyn College. The author takes pleasure in acknowledging his indebtedness to these institutions for the facilities supplied.

in two ways: (1) by a study of embryonic temperature tolerance and rate of development and (2) by breeding experiments to throw light on the inheritance of the pigment pattern.

TEMPERATURE TOLERANCE AND RATE OF DEVELOPMENT

The embryonic temperature tolerance and rate of development in amphibia is highly species specific. So far, these physiological characters have been measured in 15 species of frogs, toads, and salamanders. Although some of these species are closely related, no two are identical in these characters. For this reason the temperature tolerance and rate of development of eggs of *Rana burnsi* were compared with those of *Rana pipiens*.

Morphologically the eggs of *pipiens* and *burnsi* are indistinguishable. The color and size of the eggs as well as the structure of the jelly membranes are identical. WRIGHT and WRIGHT (1933) have found characters of this nature useful in separating species in many cases.

For practical reasons, only the upper limiting temperature was determined. Fertilized eggs of *pipiens* and *burnsi* were placed in finger bowls which were kept in water baths and cold rooms at the desired temperatures. All experiments were begun at first cleavage, and the eggs remained in the water baths until they hatched, at which time the number of normal tadpoles was counted. In every experiment from 48 to 165 (average 91.5) eggs of each female were used at any one temperature, making a total of 4940 eggs altogether. The results are given in table 1. The variability in the

TABLE 1

Normal development (in percentage) in eggs of Rana pipiens, Rana burnsi, and their hybrids at different temperatures.

EXP.	CROSS	NORMAL TADPOLES (PERCENTAGE)				
		15.0°	18.0°	27.5°	29.7°	31.8°
D	*burnsi* ♀ × *burnsi* ♂	71	84	73	18	0
D	*pipiens* ♀ × *pipiens* ♂	99	99	92	29	0
E	*burnsi* ♀ × *burnsi* ♂	95	—	20	0	—
E	*pipiens* ♀ × *pipiens* ♂	95	—	92	76	—
G	*burnsi* ♀ × *burnsi* ♂	84	—	42	12	0
G	*burnsi* ♀ × *pipiens* ♂	72	84	44	6	0
G	*pipiens* ♀ × *burnsi* ♂	—	92	44	24	0
G	*pipiens* ♀ × *pipiens* ♂	91	92	33	8	0
H	*burnsi* ♀ × *burnsi* ♂	89	75	6	0	0
H	*burnsi* ♀ × *pipiens* ♂	79	72	1	0	0
H	*pipiens* ♀ × *burnsi* ♂	78	71	63	6	0
H	*pipiens* ♀ × *pipiens* ♂	79	80	58	5	0

percentage normal development is large at the upper critical temperatures; variations of such magnitude are expected in experiments of this type. For example, in the *pipiens* controls at 29.7° the percentage normal development ranged from 5 percent in experiment H to 76 percent in experiment E. In the *burnsi* controls the percentage varied from 0 percent normal development in experiments E and H to 18 percent in experiment D. If differences do exist in the upper limiting temperature for *pipiens* and *burnsi*, they are so slight as not to be detectable by experiments of this kind. Likewise no difference can be detected between the reciprocal hybrids, or between the hybrids and the parents.

Several experiments on rate of development were conducted in which the eggs of the two species (and in some cases their reciprocal hybrids) were fertilized simultaneously and kept at constant temperatures. Comparisons of rate of development of *burnsi* and *pipiens* were made at 15° (four experiments), 18° (two experiments), 27.5° (four experiments), and 29.7° (four experiments). These failed to reveal any difference between the two forms. In two experiments in which the rate of development of the reciprocal hybrids was compared with the parents no difference could be detected. This is of interest, since in species crosses in frogs those hybrids that develop beyond gastrulation are either retarded or accelerated over the maternal rate (MOORE 1941a, 1941b).

These observations on egg size, type of jelly, rate of development, and temperature tolerance do not reveal any difference between *Rana pipiens* and *Rana burnsi*. If *Rana burnsi* proved to be a "good species" this would be surprising, but if the difference between the two is due to a single gene affecting pigmentation, as was suggested by the preliminary experiments, we would not necessarily expect embryonic differences of the type enumerated.

INHERITANCE OF THE PIGMENT PATTERN

As previously stated, the pigment pattern of the two frogs is quite dis tinct (fig. 1, 2). The black spots so characteristic of *pipiens* are nearly or completely absent in *burnsi*. The latter usually has a few spots on the hind legs, one on the elbow, and perhaps many tiny black dots in the middle of the back at the level of the pectoral girdle. Of about 50 specimens examined there was never any doubt whether a given individual was *pipiens* or *burnsi*. Confirming WEED'S experience, no intermediates were found.

The breeding experiments were conducted in the following manner: a *pipiens* ♀ and a *burnsi* ♀ were each injected with frog pituitary glands to stimulate ovulation. Two or three days later, when eggs were present in the Müllerian Duct and ready for use, one *burnsi* ♂ and one *pipiens* ♂ were killed, the gonads removed and cut into small pieces to liberate the

sperm. The necessary precautions were taken to guard against contamination by unwanted sperm. Eggs from each female were fertilized with both types of sperm. This gave four types of embryos: *burnsi* ♀ × *burnsi* ♂, *burnsi* ♀ × *pipiens* ♂, *pipiens* ♀ × *burnsi* ♂, *pipiens* ♀ × *pipiens* ♂. Hereafter these will be abbreviated B×B, B×P, P×B, and P×P, the "B" for *burnsi*, the "P" for *pipiens*, and the female preceding the male. Seven experiments were conducted (table 2). A and B were the preliminary experiments mentioned before. The tadpoles of these two were combined (the same *burnsi* male was used for both A and B females). Experiments D–H were conducted a year later than A and B. In G only B×B and P×B, and in H only B×B and B×P embryos were kept until transformation.

TABLE 2

Results of crosses between Rana pipiens and Rana burnsi. In the F_1 a segregation into burnsi and pipiens young occurs.

	BURNSI ♀ × BURNSI ♂		BURNSI ♀ × PIPIENS ♂		PIPIENS ♀ × BURNSI ♂		PIPIENS ♀ × PIPIENS ♂	
EXPERIMENT	BURNSI	PIPIENS	BURNSI	PIPIENS	BURNSI	PIPIENS	BURNSI	PIPIENS
A & B	1	2	9	9	4	2	0	7
D	123	42	33	43	31	21	0	42
E	8	3	19	11	35	54	0	40
F	31	20	40	33	8	19	0	41
G	35	7	—	—	7	6	—	—
H	26	3	10	12	—	—	—	—
Total	224	77	111	108	85	102	0	130
Expected	225½	75½	109½	109½	93½	93½	0	130
Deviation	1½	1½	1½	1½	8½	8½	0	0
St. error	7·5	7·5	7·4	7·4	6.8	6.8		

The different experiments were carried out in the same manner, so a description of one of them, D, will serve as an example for all. This experiment was begun January 1. A total of 705 embryos were kept to be raised to transformation. Of these 316 were B×B, 189 B×P, 100 P×B, and 100 P×P. These tadpoles were kept in a variety of containers, and the optimum conditions were apparently attained when 10–15 animals were in a one gallon food jar. However, in most cases the animals were more crowded than this. The diet consisted of canned spinach and cooked fresh spinach. An effort was made to change the water three times a week. The embryos were raised at room temperature (22–26°), and transformation began early in March. Even at this time there was a tremendous variation in size—best correlated with the crowded culture conditions. As a result of this, tadpoles were transforming for several months. The mortality in

experiment D, as well as in the others, was large—amounting to about 50 percent. In no case, however, was there any evidence of reduced viability as a result of hybridization. Experiment D was terminated August 11 and the few remaining tadpoles photographed and fixed for subsequent study. The number that had already transformed, together with the tadpoles fixed at this time, represented 53 percent of the original number in the B×B cross, 50 percent in B×P, 61 percent in P×B, and 50 percent in P×P.

The adult pigmentation first appears at the time of metamorphosis. The first few animals to transform showed a clear-cut segregation in the B×B, B×P, and P×B crosses. These young frogs were either spotted as in *pipiens* or non-spotted as in *burnsi* (fig. 3–6). A total of 301 transformed in the B×B experiments (table 2). Of these 224 (74.4 percent) were *burnsi* and 77 (25.6 percent) *pipiens*. In the B×P a total of 219 were secured, of which 111 (50.7 percent) were *burnsi* and 108 (49.3 percent) *pipiens*. In the reciprocal cross, P×B, 187 transformed. Of these 85 (45.5 percent) were *burnsi*, and 102 (54.5 percent) *pipiens*. Combining both types of hybrids (there is no valid reason to separate them) in a total of 406, 196 (48.3 percent) were *burnsi* and 210 (51.7 percent) *pipiens*. In the P×P cross only *pipiens* were secured. These results indicate that *Rana burnsi* differs from *Rana pipiens* by a single dominant gene and that this is carried in the heterozygous state. Thus, the B×B cross gives the expected ratio of three *burnsi* to one *pipiens*. Hybrids between *burnsi* and *pipiens*, the double recessive, show the expected 1:1 ratio. In no case is the deviation of actual from expected results more than twice the standard error (table 2). Thus the results are of statistical significance. Designating the dominant (*burnsi*) gene *B* and the recessive *b*, these crosses may be illustrated as in figure A.

P_1	burnsi Bb × burnsi Bb		burnsi Bb × pipiens bb		pipiens bb × pipiens bb
	↓		↓		↓
F_1	BB Bb Bb	bb	Bb	bb	bb
expected	75%	25%	50%	50%	100%
actual	74.4%	25.6%	48.3%	51.7%	100%

FIGURE A. Diagram of the crosses involving *Rana pipiens* and *Rana burnsi*.

ON THE QUESTION OF A HOMOZYGOUS BURNSI

To obtain the results given in table 2 a total of 13 *Rana burnsi* were used. All were heterozygous (*Bb*) for the pigment pattern gene. With the hope of discovering a homozygous (*BB*), individual further crosses were made in which *pipiens* eggs were fertilized with sperm from eight *burnsi* males. If any male was homozygous, all of its offspring would be *burnsi*. In every

case *pipiens* young appeared, indicating that the eight *burnsi* males were heterozygous. Altogether, a total of 21 *burnsi* were tested and found to be heterozygous. In other crosses, not reported in this paper, four additional *burnsi* proved also to be of the *Bb* genotype.

The collection in nature of these 25 *Rana burnsi* shown to be heterozygous raises the question of the occurrence of homozygous individuals. In this connection it is necessary to give some data on the relative numbers of *pipiens* and *burnsi* as they occur in natural populations. The two forms are found together, but the *burnsi* are in the minority. In the localities where the frogs used in these experiments were secured the dealer supplying them estimates one *burnsi* to every 99 *pipiens*. DR. GOODNIGHT informed the author that in northwestern Illinois *burnsi* likewise account for about 1 percent of the population. WRIGHT and WRIGHT (1933) found from 3–4 percent *burnsi* in Minnesota. Hardy's Formula may be used to estimate the relative frequency of the *BB* and *Bb burnsi* if certain conditions are fulfilled—that is, if the breeding population is large, if mating between *BB*, *Bb*, and *bb* frogs is random, and if the three genotypes have an equal chance of reaching maturity. Thus, in a population consisting of 1 percent *burnsi* we should expect only one *BB* for about every 400 *Bb*. If the *burnsi* are more frequent, forming 4 percent of the total population, as in Minnesota, then approximately one *BB* would occur for every 100 *Bb*. These values should be taken as rough estimates since it is not known whether or not the conditions necessary for the application of Hardy's Formula are realized. If Hardy's Formula is applicable, it is in no way remarkable that all the 25 animals tested were heterozygous.

Some observations on the *burnsi* offspring obtained in the various experiments strongly suggest that the phenotype of the *BB* and *Bb* is slightly different. In crosses between *pipiens* and *burnsi* all of the non-spotted young should be of one genotype—*Bb*. The F_1 generation of B×B, however, in addition to an expected $\frac{1}{4}$ *bb* (*pipiens*), should contain $\frac{1}{4}$ *BB* and $\frac{1}{2}$ *Bb*. A close examination of the young *burnsi* from the B×B cross (fig. 4) revealed two types that may represent the *BB* and *Bb* individuals. Most *burnsi* young have a few black spots on the hind legs, but a small number were noticed that were completely devoid of black pigment. These non-pigmented young were nearly all in the B×B cross. Some time after the experiments had been terminated the *burnsi* from experiments D, E, F, G, and H were examined and classified as pigmented (those having small black spots on the legs) or non-pigmented. The results of this study are given in table 3.[2] Except for several cases, all of the *burnsi* from B×P and

[2] The discrepancy in total numbers between tables 2 and 4 is due to the omission in table 4 of those individuals in such a poor state of preservation that accurate classification could not be made.

P×B had some black pigment (fig. 5, 6). The B×B young, on the other hand, contained nearly 30 percent non-pigmented *burnsi* (fig. 4). It is probable that these are the *BB* individuals. Theoretically we would expect the *burnsi* young from the B×B cross to consist of $\frac{1}{3}$ *BB* and $\frac{2}{3}$ *Bb*. The deviation of the actual from the expected results is 6.3 animals in a total

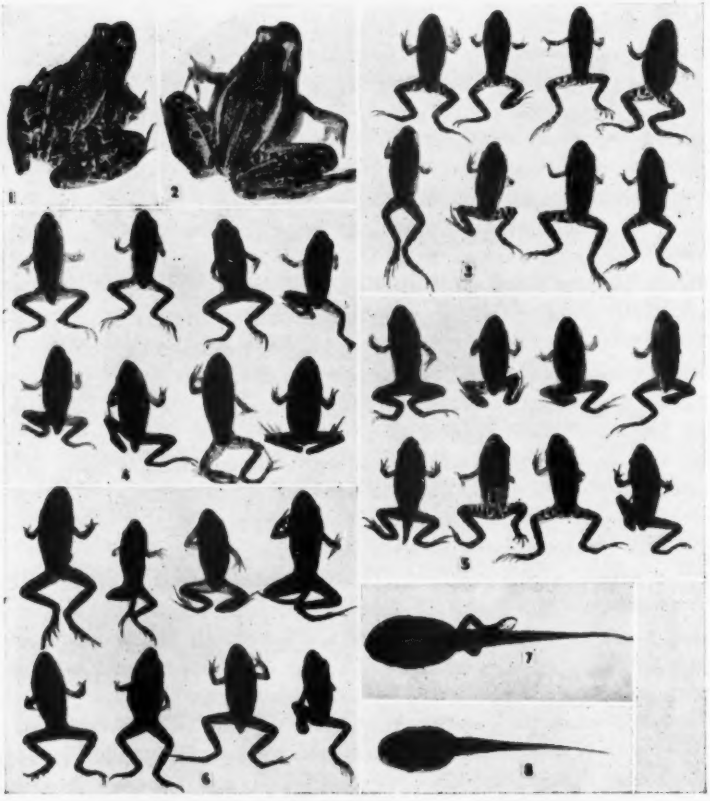


FIGURE 1.—A typical *Rana pipiens*. This was the *pipiens* female in experiment H.

FIGURE 2.—A typical *Rana burnsi*. This was the *burnsi* female in experiment H.

FIGURE 3–6.—Examples of newly transformed young from the various crosses. Fig. 3 is from *pipiens* ♀ × *pipiens* ♂. Fig. 4 is from *burnsi* ♀ × *burnsi* ♂. Fig. 5 is from *pipiens* ♀ × *burnsi* ♂. Fig. 6 is from *burnsi* ♀ × *pipiens* ♂. In fig. 5 and 6 it will be noticed that the *burnsi* young have spots on the hind legs. This is probably an indication of their heterozygous (*Bb*) nature. In fig. 4 some of the *burnsi* have spots on the hind legs (upper row) while the two at the lower left are devoid of the black leg spots. The former are probably *Bb* and the latter *BB*.

FIGURES 7–8.—The two types of tadpoles, spotted and non-spotted. The spotted tadpoles in most cases give rise to *pipiens* young, and the non-spotted tadpoles in most cases give rise to *burnsi* young.

TABLE 3

Classification of burnsi young from various crosses as to those with some black pigment and those devoid of black pigment.

EXPERIMENT	BURNSI ♀ × BURNSI ♂		BURNSI ♀ × PIPIENS ♂		PIPIENS ♀ × BURNSI ♂	
	PIGMENT	NO PIGMENT	PIGMENT	NO PIGMENT	PIGMENT	NO PIGMENT
D	57	32	26	2	24	0
E	8	0	15	0	27	0
F	15	8	32	0	4	1?
G	23	8	—	—	6	0
H	18	3	9	0	—	—
Total	121	51	82	2	61	1?
%	70.3	29.7	97.6	2.4	98.4	1.6

of 172. Since the standard error is 6.1, it is clear that the results are significant. These observations indicate that the *burnsi* gene is not completely dominant, and the small amount of black pigment on most *burnsi* is an indication of their heterozygosity.

LARVAL DIFFERENCES BETWEEN PIPIENS AND BURNSI

By the time the tadpoles reached 40 mm (and perhaps earlier) it became apparent that they were not uniform in their pigmentation. In the P×P cross all of the tadpoles were covered with fine black spots (fig. 7). The B×B, B×P, and P×B crosses, however, consisted of some with spots and others nearly or completely devoid of spots (fig. 8). When the tadpoles were two months old, those in the D (except some B×B and B×P) and E experiments were classified as spotted and non-spotted tadpoles. Thereafter these two groups were kept in separate containers. The variation in

TABLE 4

The relation between larval and adult pigmentation. In general, spotted tadpoles transform as pipiens, and non-spotted tadpoles transform as burnsi.

EXP.	F_1	BURNSI ♀ × BURNSI ♂		BURNSI ♀ × PIPIENS ♂		PIPIENS ♀ × BURNSI ♂		PIPIENS ♀ × PIPIENS ♂	
		SPOTTED TADPOLES	NON-SPOTTED TADPOLES	SPOTTED TADPOLES	NON-SPOTTED TADPOLES	SPOTTED TADPOLES	NON-SPOTTED TADPOLES	SPOTTED TADPOLES	NON-SPOTTED TADPOLES
D	*pipiens*	9	3	20	0	20	1	42	0
	burnsi	0	28	0	18	13	18	0	0
E	*pipiens*	3	0	10	1	51	3	40	0
	burnsi	0	8	1	18	7	2	0	0

size at the time of classification was considerable; in a few cases it was difficult to assign small tadpoles to the correct class, and some errors were made, which became apparent when these small ones increased in size, but no change was made from the original classification. The results are given in table 4. Of the tadpoles originally classed as spotted, 90.3 percent gave rise to *pipiens* and 9.7 percent to *burnsi*. Of those classed as non-spotted, 93.7 percent gave rise to *burnsi* and 6.3 percent to *pipiens*. The adult pigment differences are thus heralded in the larval stage. The exceptions (*burnsi* from spotted tadpoles and *pipiens* from non-spotted tadpoles) can be explained in part by incorrect classification of very young tadpoles. However, this cannot account for all the exceptions, since in a few cases definitely spotted individuals transformed into *burnsi* and others devoid of larval spots gave rise to *pipiens*. The amount of larval pigmentation does vary. In experiment E crosses involving *pipiens* eggs resulted in tadpoles with much heavier pigmentation than in the other experiments. Thus in experiment E the classification of P×B tadpoles was as heavily spotted and lightly spotted (instead of non-spotted) tadpoles. It is possible that if classification were postponed until the tadpoles were 50 mm, an accuracy of 98 percent could be realized.

CONCLUSIONS

Rana burnsi differs from *Rana pipiens* by one dominant gene that influences pigmentation. Twenty-five animals have been tested and found to be heterozygous for the mutant in question. *Rana burnsi* should not have the status of a species or subspecies but should be reduced to synonomy with *Rana pipiens* and be referred to as the "burnsi mutant."

LITERATURE CITED

DICKERSON, M. C., 1906 The frog book. New York: Doubleday, Page.

KELLOGG, R., 1932 Mexican tailless amphibians in the United States National Museum. Bull. U. S. Nat. Mus. **160**.

MOORE, J. A., 1941a Developmental rate of hybrid frogs. J. Exp. Zool. **86**: 405–422.

1941b Developmental rate of hybrids between *Rana pipeins* and *Rana sphenocephala*. Proc. Soc. Exp. Biol. N. Y. **47**: 207–210.

STEJNEGER, L., and T. BARBOUR, 1939 A check list of North American amphibians and reptiles. Cambridge, Mass.: Harvard University Press.

WEED, A. C., 1922 New frogs from Minnesota. Proc. Biol. Soc. Wash. **35**: 107–110.

WRIGHT, A. A., and A. H. WRIGHT, 1933 Handbook of frogs and toads. Ithaca, N. Y.: Comstock Publishing Co.

THE DEVELOPMENT OF EYE COLORS IN DROSOPHILA MELANOGASTER. FURTHER STUDIES ON THE MUTANT CLARET[1]

C. W. CLANCY
Stanford University[2]

Received January 22, 1942

INTRODUCTION

THESE studies were undertaken in order to repeat and supplement the experiments of BEADLE and EPHRUSSI (1936, 1937) and EPHRUSSI and BEADLE (1936b) which provided the evidence upon which these authors postulated the existence of ca^+ substance as one of three specific diffusible substances assumed to be necessary for the differentiation of wild type eye color in *Drosophila melanogaster*.

The first portion of this paper is primarily concerned with a re-examination of this evidence and the presentation of new data bearing on the question of whether or not ca^+ substance exists as an entity distinct from v^+ and cn^+ substances. [For information concerning the chemical identification of v^+ hormone see BUTENANDT, WEIDEL, and BECKER (1940), TATUM and BEADLE (1940), and TATUM and HAAGEN-SMIT (1941).] This problem has led to the consideration of other aspects of the differentiation of eye color involving the mutant claret. One of these aspects is the effect of the mutation $ca^+ \rightarrow ca$ on the quantity of the pigment components making up the eye color of *D. melanogaster*. A second portion of this paper presents in connection with data bearing on the latter problem an outline of a general method for the extraction and measurement of the eye-color pigments of Drosophila.

Doubt concerning the existence of ca^+ substance has been expressed by GOTTSCHEWSKI and TAN (1938) and by BEADLE, ANDERSON, and MAXWELL (1938). Recently, LUERS and STUBBE (1940) have sought to determine the content of free ca^+ substance in the blood of *D. melanogaster* and two other species of Drosophila. Their results will be discussed later.

Evidence has now accumulated which invalidates the original argument upon which the existence of ca^+ substance was based. This negating evidence consists, in part, in the correction of certain observational errors made in the original experiments. The recognition of these errors permits a different interpretation to be placed upon the results of certain optic-disc transplantations involving, especially, wild type and the mutants claret and vermilion.

[1] Part of a thesis submitted to the School of Biological Sciences of STANFORD UNIVERSITY in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Now at the UNIVERSITY OF OREGON, Eugene.

When *ca*$^+$ substance was postulated, the following transplantation results were considered valid[3] (See BEADLE and EPHRUSSI 1936, 1937): (1) + in *ca*→*ca*, (2) *v* in *ca*→*v*, (3) *cn* in *ca*→*cn*, (4) + in *v*→+, (5) + in all eye color mutants except claret→+.

Based on this interpretation of the color of the implants, the argument for the existence of *ca*$^+$ substance is as follows: A wild type optic disc evidently requires a substance from some other part or parts of the fly's body in order to develop wild type pigmentation. This substance is lacking in a claret host. Since a vermilion fly lacks both *v*$^+$ and *cn*$^+$ substances, but, as a host, allows a wild type optic disc to develop wild type pigmentation, vermilion must contain the substance lacking in the claret host. Because transplants of vermilion and cinnabar optic discs were not modified toward wild type when transplanted to claret hosts, *ca*$^+$ substance was assumed to be a precursor to the formation of *v*$^+$ substance in the reaction chain, (*ca*$^+$ substance)→*v*$^+$ substance→*cn*$^+$ substance.

It was soon necessary to adapt this conception to a new observation—namely, that a wild type optic disc grown in a claret host was not always phenotypically claret but occasionally approached wild type in color. Specific experiments designed to clarify the meaning of this observation were carried out by EPHRUSSI and BEADLE (1936b) as a result of which they interpreted the color of wild type discs grown in claret hosts to be phenotypically like claret only when the transplantations were made within 80 hours after egg-laying (at 25°C). If the same transplantations were made shortly before puparium formation (about 106 hours after egg-laying), the resulting implant was phenotypically close to wild type. They interpreted these results as indicating the presence of a critical period in the larval life of a wild type fly, between 80 and 106 hours after egg-laying, during which *ca*$^+$ substance moved from the body to the eye. They inferred that after this critical period the transplantation of a wild type optic disc to a host unable to supply this substance would not necessarily modify the normal course of pigment development in the disc.

Along with these results EPHRUSSI and BEADLE (1936b) also reported that additional and more sensitive tests showed that the claret host does

[3] Standard genetic symbols for the mutants of *Drosophila melanogaster* are used; for their significance consult MORGAN, BRIDGES, and STURTEVANT (1925). A list of the races mentioned along with their symbols follows: +, wild type (an inbred stock of +Oregon-R was used in all experiments); *bw*, brown; *ca*, claret; *car*, carnation; *cd*, cardinal; *cm*, carmine; *cn*, cinnabar; *g*^{2}, garnet-2; *p*, pink; *rb*, ruby; *st*, scarlet; *se*, sepia; *v*, vermilion; *w*, white; *w*^{e}, eosin.

In addition, a shorthand notation employed by BEADLE and EPHRUSSI (1937) for designating transplantations of optic discs is also used. For example, *v* in +→+ means that an operation has been performed in which a genetically vermilion optic disc has been transplanted to a wild type host, has differentiated, and upon comparison with implants from the control operations, (*v* in *v*) and (+ in +), shows no essential difference in its pigmentation from that found in the implant from the (+ in +) control operation. For details concerning the technique of transplantation in Drosophila see BEADLE and EPHRUSSI (1936) and EPHRUSSI and BEADLE (1936a).

not completely lack v^+ and cn^+ substances as originally supposed. The bearing of these new observations on the general scheme relating the three postulated diffusible substances was referred to in their paper, but a detailed discussion was not given, possibly because data available at that time were considered inadequate to merit such a discussion.

Shortly afterward, EPHRUSSI and BEADLE (cited in EPHRUSSI and CHEVAIS 1938) found that wild type optic discs grown in vermilion hosts were not quite autonomous in their pigment development. EPHRUSSI and CHEVAIS (1938), who repeated this transplantation, pointed out that the difference in pigmentation between wild type implants grown in vermilion hosts and the control implants was slight. They accounted for this "limited self-differentiation" of the wild type optic disc as being due to the disc's inability to produce enough v^+ hormone to completely satisfy its pigment requirement.

Pertinent information on hand when the present studies were begun may be summarized by re-stating the transplantations listed above in the following manner:

(1) + in *ca* → *ca* (before critical period)
(1) + in *ca* → Close to + (after critical period)
(2) *v* in *ca* → Intermediate between *v* and +
(3) *cn* in *ca* → Intermediate between *cn* and +
(4) + in *v* → "limited self-differentiation" of +

Data bearing on the fifth member of the series of transplantations listed earlier are presented in table 1.

BEADLE, ANDERSON, and MAXWELL (1938) took note of the inconsistencies in the evidence for ca^+ substance as indicated in the following footnote quoted from their paper: "Experiments as yet unpublished suggest that the so-called 'ca^+—substance' of Drosophila does not exist as a distinct substance but what may be called the 'claret effect' is one aspect of the action of either v^+ or cn^+ substance."

The unpublished data mentioned in this footnote were turned over to the writer by PROFESSOR G. W. BEADLE in the summer of 1939 and are presented in the experimental portion of this paper (see table 1).

MATERIAL AND METHODS

The wild type stock and the mutants used are listed in footnote 3. All flies were cultured at 25°C.

Transplantation operations were performed by the method of EPHRUSSI and BEADLE (1936a), and in all cases where semi-quantitative estimations of v^+ and cn^+ hormones were made, vermilion-brown and cinnabar-brown test larvae were used as described by TATUM and BEADLE (1938).

In testing whole pupae for content of v^+ hormone, a method for securing

TABLE 1

*Differentiation of wild type optic discs implanted into various eye-color mutant hosts. Under the heading "number of individuals" in this and certain other tables to follow, the four sex combinations and total are given in the order: female in female, female in male, male in female, male in male, and total.**

EXP. NO.	HOST	HOURS AFTER EGG-LAYING	TIME TO PUPARIUM FORMATION (HRS.)	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANTS
71	*ca*	70–74	38–39	6-12-0-0:18	Lighter than +; not *ca*
72†	*ca*	67–71	41–42	4-1-0-0: 5	2 ♀♀ darker than 71; 2 ♀♀ and 1 ♂ about same as 71
73	*ca*	66–70	44–45	6-4-0-0:10	Lighter than +
74	*ca*	90–93	20–21	4-5-0-0: 9	Same as above (73)
75	*ca*	108–111	2–3	7-3-0-0:10	Some darker than above, but not +
76‡	*ca*	66–70	—	2-3-0-0: 5	Very close to 73
77§	*ca*	66–70	—	1-1-0-0: 2	1 disc same as lightest +; but darker than 73. 1 disc lighter than +; but darker than average of 73
78	*v*	68–72	41–43	10-2-4-6:22	Slightly lighter than +
79	*v*	102–105	8–9	5-0-4-1:10	Same as 78
80	*v*	68–71	45–46	1-4-3-3:11	Slightly lighter than +
81	*ca*	69–72	44–45	1-3-1-3: 8	Same as 80
82	*car*	71–74	—	9-3-6-3:21	Lighter than +
83	*cm*	71–73	45–46	2-6-3-3:14	Not *cm*; *ca*-like but not the same
84	g^2	67–70	45–46	2-3-0-0: 5	Lighter than +; not g^3
85	g^2	71–73	42–43	0-0-3-5: 8	Same as 84
86	*p*	70–74	—	6-8-3-5:22	Lighter than +; not *p*
87	*rb*	69–72	42–43	2-4-4-3:13	*ca*-like; close to *rb*
88	*rb*	94–96	18–19	2-4-3-7:16	Same as 87
89	*cd*	64–68	45–46	1-3-1-0: 5	Same as + control
90	*cn bw*	70–75	42–43	10-2-4-7:23	Same as + control
91	*ma*	64–67	50–51	7-2-0-0: 9	Same as + control
92	*se*	68–71	—	5-3-0-0: 8	Same as + control
93	*se*	75–78	—	0-0-4-4: 8	Same as + control
94	*w*	70–73	—	6-0-0-3: 9	Same as + control (possibly lighter??)
95	*w*	92–98	18–27	5-6-0-0:11	Same as + control
96	*sl*	68–73	44–46	6-1-4-4:15	Slightly lighter than +, but close

* Data kindly furnished by PROFESSOR G. W. BEADLE, STANFORD UNIVERSITY.
† Two + malpighian tubes inplanted 24–26 hours after optic disc transplantation.
‡ One + fat body implanted 42–44 hours after optic disc implantation.
§ Four + malpighian tubes implanted 43–44 hours after optic disc implantation.

bacteriologically sterile larvae (TATUM and BEADLE 1939) was adapted to the feeding technique of BEADLE and LAW 1938). The procedure was as follows: Eggs of wild type and claret were collected over a 24-hour period and allowed to develop on an excess of food. Thirty-six to 60 hours after

puparium formation the pupae were washed, counted, and killed by immersion in boiling water for 45 seconds. The pupae were then transferred aseptically to sterilized 8-dram shell vials and thoroughly mashed. Two drops of a thick suspension of dried brewer's yeast in 2 percent sucrose solution were added to this pulp in order to ensure an excess of food, to inhibit any starvation effect, and to prevent dessication. Bacteriologically sterile vermilion-brown test larvae, 72–76 hours old, were transferred to these vials and on emergence classified as to eye-color modification by comparing them with the genetic eye-color standards of TATUM and BEADLE (1938).

A method for the extraction and measurement of the eye-color pigments is described later in this paper.

CONCERNING CA$^+$ SUBSTANCE

Analysis of data received from G. W. Beadle, Stanford University

BEADLE re-examined the pigmentation of wild type optic discs recovered from transplantations to claret and various other eye-color mutant hosts. His results are summarized in table 1, where the experiments are arranged in three groups.

Transplants of wild type optic discs to claret are listed in the upper section. In all cases the implant was "lighter than wild type" in color and not-claret. The ages tested range from 45 to 2 hours before puparium formation. These results are at variance with those obtained by EPHRUSSI and BEADLE (1936b) in that the phenotype of a wild type implant grown in a claret host could not be construed as claret. The evidence at hand shows that the earlier interpretation of the color of (+ in *ca*) implants was at fault in that the "lighter than wild type" appearance of the implant in some cases was judged to be phenotypically claret.

In addition to the ordinary optic-disc transplantations, three experiments were performed in which either wild type Malpighian tubes or wild type fat-bodies were implanted along with the optic disc (see legend and footnotes in table 1) in order to determine whether the optic disc would approach wild type pigmentation when these organs, known to be sources of v^+ hormone, were present. In the case of an added fat-body, two of the optic discs were darker than those from the (+ in *ca*) controls. With one pair of Malpighian tubes the result was doubtful. Four added Malpighian tubes clearly affected the color of the implant but did not make it so dark as most of the (+ in +) controls. These results indicate that if the amount of v^+ hormone in the claret host is increased, the wild type implant is able to use it.

In the middle section data are given for transplants of wild type discs, first to vermilion alone, and then to vermilion and claret for direct comparison of the implants. No differences in pigmentation could be detected

between implants from either of these hosts, and the phenotype was "lighter than wild type."

In the lower section the mutants used as hosts to wild type optic discs are grouped into two classes—(1) those in which the implant attained full wide type pigmentation and (2) those in which the implant failed to do so. Since the operations were done at different times, all possible direct comparisons could not be made. Control implants of host constitution were available, however, and in all instances were distinguishable from the wild type implants. Presumably, the implants designated as "lighter than wild type" were similar to each other and to those from claret and vermilion hosts. Thus, it was found that certain eye-color mutants, carnation, carmine, garnet-2, pink, and ruby gave results essentially similar to claret —that is, the "claret effect" was not limited to claret.

The other group of eye-color mutants, cardinal, maroon, sepia, white, and the double recessive, cinnabar-brown, were not distinguishable from wild type as hosts to a wild type implant. The result with scarlet was doubtful.

All of the eye-color mutant hosts here giving the "claret effect" were known to contain less v^+ hormone than wild type (BEADLE and EPHRUSSI 1937). In view of this correlation the original argument for the existence of ca^+ substance is no longer tenable.

TRANSPLANTATION OF (+ in *ca*) AND (+ in +) INVOLVING HOSTS AND DONORS OF DIFFERENT AGES

There still remained some question about (+ in *ca*) implants attaining a pigmentation phenotypically close to wild type as reported by EPHRUSSI and BEADLE (1936b). A series of transplantations of (+ in *ca*) and (+ in +) in which host and donor were of the same age was made both to check on previous observations and to compare with those from the same combinations in which host and donor were of different age. The data are contained in table 2. In all the equal-age combinations the wild type implant recovered from the claret host was lighter red in color than the control implant of comparable age. These implants were simultaneously compared with the age-difference combinations. The latter, along with those equal-age combinations with which they were directly compared, are distinguished in table 2 by a code indication of the age relation between host and donor. Those transplantations which have no code indication are equal-age combinations not used in these comparisons but made to complete the series at close age-intervals up to puparium formation. The specific comparisons made in each case are stated in the last column of the table. In addition, figure 1 illustrates graphically the transplantation combinations and cross-comparisons made in specific experiments.

Within the limits of the age-differences tested, a wild type optic disc

TABLE 2

*Transplants of wild type optic discs to claret and wild type hosts. In column four under the heading "code," Y = young, and O = old. For further explanation see text. The numbers in parentheses give the mean age of the larvae at the time of transplantation.**

EXP. NO.	CONSTITUTION AND AGE IN HOURS OF DONOR	HOST	CODE	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANTS (COMPARISONS)
513	+(93)	+(93)	Y in Y	2-0-0-0:2	Same as 508, 510, and 512, but darker than 514
503	+(103)	+(103)	O in O	2-0-0-0:2	Darker than 500 and 501
508	+(113)	+(113)	O in O	2-2-1-2:7	Same as 513, 510, and 512
525	+(121)	+(121)	—	5-0-0-0:5	Darker than 524; different from *ca*
510	+(91)	+(114)	Y in O	2-0-0-0:2	Same as 512 and 508
512	+(115)	+(92)	O in Y	0-2 0 0:2	Same as 510 and 508
514	+(94)	*ca* (94)	Y in Y	2-0-0-0:2	Lighter than 510, 512, and 513; same as 515
500	+(96)	*ca* (96)	—	0-0-1-0:1	Lighter than 503;
501	+(97.5)	*ca* (97.5)	—	1-0-0-0:1	Same as 500
517	+(115)	*ca* (115)	O in O	1-0-0-0:1	Lighter than +; same as 516
535	+(119)	*ca* (119)	—	6-1-0-0:7	Lighter than +
524	+(120)	*ca* (120)	—	3-0-0-0:3	Lighter than 525; different from *ca*
530	+(122)	*ca* (122)	—	1-3-0-0:4	Lighter than +
547	+(124)	*ca* (124)	—	3-1-0-0:4	Lighter than +
516	+(90)	*ca* (114)	Y in O	4-0-0-0:4	Same as 517
515	+(117)	*ca* (94)	O in Y	2-0-0-0:2	Same as 516

* The mean age was calculated from the mean time of the egg-laying period to the mean time of the transplantation period. In this and the following tables in which the ages of larvae are given, the maximum deviation from the mean is ±2.5 hours.

grown in a wild type host is readily distinguishable from one grown in a claret host regardless of the age combination of the latter with respect to donor and host. Since the age of the youngest larvae involved in these transplantations exceeded by 10 to 15 hours that point which EPHRUSSI and BEADLE (1936b) assumed to be the beginning of the critical period for the movement of ca^+ substance from body to eye, the constant difference between (+ in +) and (+ in *ca*) implants cannot be explained by their hypothesis.

COMPARATIVE MEASUREMENTS OF V^+ HORMONE IN WILD TYPE AND CLARET

As mentioned above, claret along with a number of other eye-color mutants is characterized by having a reduced amount of v^+ hormone as compared to wild type. Since this fact is here used to account for the so-called "claret effect," some idea of the magnitude of the difference between claret and wild type in this respect seemed desirable. Measurements of the amount of v^+ hormone released by transplants of Malpighian tubes and fat-bodies were therefore made. In addition, crushed whole pupae of wild

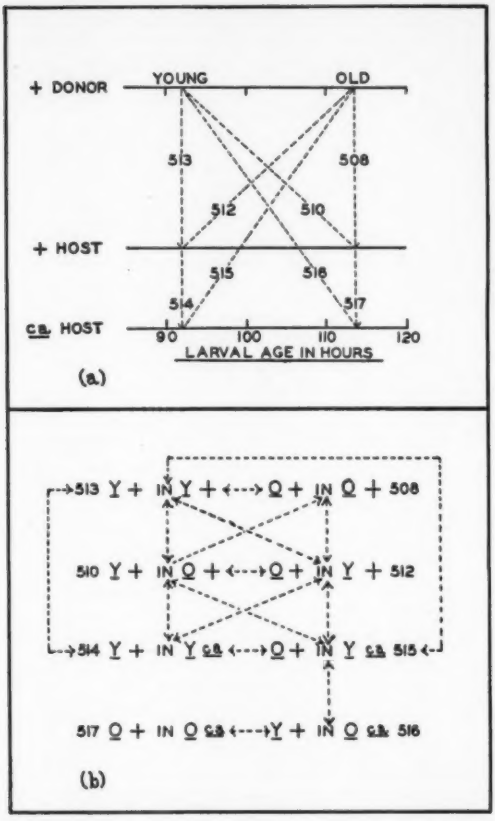


FIGURE 1.—(a) Diagram indicating age relations between host and donor for the transplantations recorded in table 2.—(b) Diagram showing the comparisons made between implants in the experiments recorded in table 2; see table 2 for significance of abbreviations.

type and claret were tested as to their content of v^+ hormone by feeding them to vermilion-brown test larvae.

(a) *Transplantation of Malpighian tubes and fat-bodies*

BEADLE (1937a, 1937b) tested both the Malpighian tubes and fat-bodies of wild type and most of the eye-color mutants for v^+ and cn^+ hormones. His tests were made before a semi-quantitative method for the evaluation of hormone activity was available. Results of the present tests are recorded in table 3.

In terms of unit hormone activity (TATUM and BEADLE 1938), wild type Malpighian tubes are some 19 times as active as those of claret.

TABLE 3

Tests for the release of v^+ hormone. The units of hormone per implant are calculated on the basis of the maximum color modification attained by the vermilion-brown test animal.

IMPLANT	NUMBER OF INDIVIDUALS	MODIFICATION OF THE HOST'S EYES			
		COLOR VALUES		UNITS PER IMPLANT	RATIO + TO *ca*
		MEAN	RANGE		
Malpighian Tubes:					
ca	5-2-0-0: 7	0.34	(0.3–0.5)	0.65	19.2
+	6-2-1-2: 11	3.72	(3.5–4.0)	12.50	
*Fat Bodies:					
ca	7-3-3-2: 15	2.4	(1.8–2.5)	3.5	2.3
+	3-2-0-1: 6	3.1	(2.5–3.5)	8.2	

* The fat-body transplantations were made at the UNIVERSITY OF OREGON, and the work was assisted by a grant-in-aid from the General Research Council, Oregon State System of Higher Education.

When fat-bodies are considered on the same basis, the difference between claret and wild type, although in the same direction, is not so striking. Wild type fat-bodies release slightly more than twice as much v^+ hormone as do those of claret. From this it is evident that the Malpighian tubes and fat-bodies are affected differentially in their relation to v^+ hormone by the mutation $ca^+ \rightarrow ca$.

(b) *Feeding experiments with pupae of + and ca*

The details of the procedure employed in this test are given above, under the section Materials and Methods, and the results are recorded in table 4.

If either mean color-values or maximum units of hormone are used as a basis of comparison, the ratio of wild type to claret is 2.8. It may be inferred from this rather crude test that between 36 and 60 hours after puparium formation a wild type pupa contains about three times as much v^+ hormone as a claret pupa of the same age.

Transplants of (+ in *ca*) *and* (+ in *w*; *ca*)

It is known that a wild type optic disc can produce some v^+ hormone itself and that this amount is not enough for its total pigment requirement (E. B. CLANCY 1940). BEADLE's data (see table 1) show that its pigment is still only partially differentiated when it is grown in hosts (*ca*, *cm*, et al.) containing reduced amounts of v^+ hormone. Moreover, BEADLE'S experiments (see footnotes table 1) in which wild type Malpighian tubes or fat-bodies served as additional sources of v^+ hormone indicate that complete pigment differentiation is possible in an otherwise claret milieu. A possible objection to the latter experiments is that the sources of extra v^+ hormone

TABLE 4

Data showing the effect on the eye color of vermilion-brown test anima s of feeding crushed pupae of wild type and claret.

EXPER. NO.	VIAL NO. AND STOCK	NUMBER OF INDIVIDUALS ♀♀	♂♂	TOTAL	COLOR VALUES MEAN	RANGE	MAXIMUM UNITS HORMONE
721a	(1) *ca*	5	4	9	0.62	(0.5–0.8)	0.85
	(2) *ca*	2	7	9	0.52	(0.5–0.6)	0.70
	(3) *ca*	7	6	13	0.51	(0.3–0.7)	0.78
	(4) *ca*	2	6	8	0.40	(0.3–0.6)	0.70
722a	(1) *ca*	5	2	7	0.80	(0.5–1.0)	1.00
	(2) *ca*	2	2	4	0.60	(0.4–0.7)	0.78
	(3) *ca*	3	3	6	0.58	(0.5–1.c)	1.00
		26	30 =	56	m=0.58		m=0.83
721b	(1) +	4	4	8	1.80	(1.5–2.1)	2.50
	(2) +	2	6	8	1.90	(1.5–2.0)	2.30
	(3) +	3	3	6	1.20	(0.8–1.8)	2.00
	(4) +	4	3	7	2.00	(———)	2.30
722b	(1) +	7	3	10	1.50	(0.8–2.1)	2.50
	(2) +	4	3	7	1.70	(1.0–2.1)	2.50
		24	22 =	46	m=1.68		m=2.35
Control	*v; bw*	4	3 =	7	0.0		

TABLE 5

Transplants of wild type optic disc to claret and white-claret hosts. All transplantations were made shortly before puparium formation.

EXPER. NO.	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANTS
528	*w*	4-2-0-0:6	Same as 531; darker than 530
529	*w*	0-0-4-2:6	Same as 531; darker than 530
530	*ca*	1-3-0-0:4	
531	+	2-1-0-0:3	
534	*w; ca*	3-2-0-0:5	Not so dark as 536; darker than 530
535	*ca*	6-1-0-0:7	Lighter than 534
536	+	2-3-0-0:5	
546	*w; ca*	4-3-0-0:7	4 lighter than 548; 3 same as 548*
547	*ca*	3-1-0-0:4	Lighter than either 546 or 548
548	+	2-2-0-0:4	

* See text for further notes on this experiment (546 and 548).

may also supply ca^+ substance. To obviate such criticism, transplants of wild type discs to white-claret and claret were compared on the assumption that by introducing the white gene the effective concentration of v^+ hormone would be increased by whatever amount is ordinarily utilized in claret pigmentation. This assumption is an adaptation of principles worked out by EPHRUSSI and CHEVAIS (1937, 1938) in their studies on the relations between production, utilization, and release of diffusible substances in eye-color development.

Transplants were first made to white, claret, and wild type. Implants (see table 5) from (+ in *w*) and (+ in +) were indistinguishable. Implants from (+ in *w*; *ca*) were darker than those from (+ in *ca*), but not so dark as those from white or wild type hosts. An exception, in which the implants were as dark as those from wild type hosts, was found in experiment 546 (see table 5). In this experiment all donor discs were derived from wild type females. The protocol is as follows:

(+ ♀ discs in *w; ca*)

	Hosts	*Implants*
(a)	3 ♀♀	medium-sized discs, dark in color
	1 ♂	small-sized disc, dark
(b)	1 ♀	large-sized disc } All lighter than above.
	2 ♂♂	large-sized discs }

The discs from (a) were indistinguishable from those of experiment 548 (+ in +), while those from (b) were slightly lighter in color. All of the latter were darker, however, than implants from experiment 547 (+ in *ca*). It appears obvious that the size of the implant to some extent determines the intensity of color attained in a given hormone environment, but this does not detract significance from the fact that some wild type implants attained full wild type pigmentation in white claret hosts.

To explain these results it is assumed that, because it completely blocks pigment formation, the presence of the white gene in the double recessive white-claret raises the concentration of v^+ hormone to a level where its utilization by the wild type implant in forming additional brown pigment may be detected by this method of observation. The amount of v^+ hormone made available to the implant by this means is not sufficient for the attainment of full wild type pigmentation unless the implant is smaller than usual for these transplants.

PIGMENT MEASUREMENTS

After the above experiments were under way an opportunity to extend them arose when equipment became available for measuring eye-color pigment. The studies reported in this section represent part of an attempt to

characterize the action of the claret gene in relation to the general scheme of eye-color development outlined by BEADLE and EPHRUSSI (1936), EPHRUSSI and CHEVAIS (1938), and BEADLE and TATUM (1941).

Attention is called to the pioneering work of SCHULTZ (1932, 1935), MAINX (1937, 1938), and BECKER (1939) with Drosophila eye pigments which showed that the + pigment consists of two types of pigments: the brown and the red components and thus formed the working basis upon which the methods employed here were devised.

The action of the mutant gene claret on the pigment components of the eye was studied (1) by comparing the amount of red and brown pigment contained in the eyes of wild type and claret and (2) by noting the kind and quantity of pigment present in the eyes of double recessive stocks made up of claret plus some other eye-color gene known to block the formation of either the red or brown pigment component. The latter technique is essentially the same as that used by MAINX (1938) in his more general analysis of gene action.

Method for extraction and measurement of pigment

Eggs of the desired stocks were collected over 24-hour egg-laying periods on food contained in small metal trays. The larvae when 30 to 48 hours old were transferred (100 to 125 larvae per bottle) to culture bottles containing the standard medium enriched with dried brewer's yeast. It was hoped that this procedure would provide optimal growth conditions (at 25°C and a relative humidity of 70–80 percent) and that a characteristic maximal size would be attained which might be considered fairly constant for flies of a given genetic constitution. In the early experiments no other attempt to control the size factor was made. In some of the later experiments an indirect check was made by weighing male sibs of the flies used for extracts. Males were used instead of females because of the variable weight of developing eggs in the latter. All extracts were made from the heads of females, and care was taken to secure a random sample of the flies hatching from any given series of bottles. Decapitation was performed 24 to 48 hours after emergence, and an effort was made to use only uninjured heads. Each extract was ordinarily prepared from 100 heads. In a few instances where the pigment content was known to be low, 200 to 500 heads were used. In all cases, however, the values recorded in tables 6 to 10, inclusive, are based either directly on the pigment extracted from 100 heads contained in 10.0 cc of solvent, or have been calculated to that base.

The measurements of dissolved pigment were made with an Evelyn Photo-electric Macro-colorimeter manufactured by the Rubicon Company, Philadelphia. They are recorded in terms of "Photometric Density," a value directly proportional to the relative concentration of pigment, as

defined in the manual accompanying this instrument. Absorption curves for solutions of the eye pigments were obtained from DR. E. L. TATUM (unpublished) and a filter selected which allowed passage of wave lengths of light corresponding to the region of maximum absorption. The particular filter used with the above colorimeter bears the code number, 440, indicating in millimicrons the wave length of maximum transmission. Dilution curves for solutions of the pigments in the solvents described below were supplied by PROFESSOR G. W. BEADLE (unpublished). When necessary, extracts were diluted to a concentration range where the relation between "Photometric Density" and concentration is strictly linear.

In preparing pigment extracts from the heads of wild type and claret the aim was to remove the water-soluble red pigment first and then to remove by means of a different solvent the relatively insoluble brown pigment. This was known to be soluble at room temperature in 2N hydrochloric acid (BECKER 1939) and in an anhydrous solution of 1.0 percent H Cl in methyl alcohol (E. L. TATUM, unpublished). All extractions of the brown pigment reported here were made at room temperature with the latter solvent. For convenience it will be referred to as "Solvent B."

Various attempts were made to quantitatively extract the red pigment from intact whole heads by treating them with water. None of these trials was successful, and the details need not be related here. The solvent finally used for removing the red pigment was a solution of 30 percent ethyl alcohol acidified with H Cl to pH 2.0. When this solvent was tested on the heads of vermilion, scarlet, and cinnabar flies, it removed the pigment readily and completely. It will be referred to as "Solvent A."

When Solvent A was originally tested on the heads of the mutant brown, it was concluded (unjustifiably, it now appears) that the brown pigment characterizing the eye color of this mutant was quite insoluble in this solvent. However, after soaking the heads for almost two months in Solvent A, a value was obtained which corresponds to about 15 percent of the total amount of brown pigment extractable with Solvent B. For this reason the method of double-extraction described below for the removal of pigment from wild type and claret is definitely open to criticism. Corrections in the pigment values obtained for wild type and claret could be made, utilizing the maximum value obtained for the control. This has not been done, because critical evidence necessary to justify such a procedure is lacking.

The procedure in the case of wild type and claret was to place the intact heads immediately after decapitation into a small homoeopathic vial containing 2 to 3 cc of Solvent A. Several days later (12 to 38 days is the range for the experiments reported) the extract containing most of the pigment was quantitatively removed and a fresh supply of solvent added. This was

removed after a few days, combined with the first extract, filtered under reduced pressure through ground glass, and brought up to 10.0 cc volume with the pure solvent. The relative amount of pigment contained in this volume of extract was then measured either directly or after dilution to an appropriate concentration as explained above. After this treatment for the removal of the red pigment with Solvent A, the heads were rinsed for a few seconds in absolute methyl alcohol, and 2 to 3 cc of Solvent B were added for removal of the residual brown pigment. Shortly before taking measurements, the brown pigment was reduced by the addition of a few crystals of sodium hydrosulfite.

The extraction of brown pigment was usually accomplished in two to three days, although in some experiments the heads were exposed to the solvent for a longer time. Comparable experiments indicated that the brown pigment solutions were quite stable at room temperature, permitting considerable latitude in time for handling the extracts. This is not true for solutions of the red pigment at room temperature. A collateral experiment carried out during the time that most of the pigment measurements were made showed that the percentage concentration of pigment decreased with time. Comparable solutions of red pigment were kept at room temperature, at 25°C, and in the refrigerator (6° to 8°C) and measured from time to time during the course of 68 days. The data are contained in table 11, and self-explanatory curves are given in figure 2. Here again, corrections in the original measurements have not been made because of the lack of pertinent data. In all cases involving the red pigment the lapse of time (in days) between decapitation and measurement is indicated in the tables.

After the heads of vermilion, scarlet, and cinnabar are extracted with Solvent A, they appear white when examined under a low power binocular microscope, indicating that practically complete removal of the pigment has been effected. The same is true after extraction of the brown pigment with Solvent B from the heads of brown and brown-claret. In contrast, a residual pinkish color may be noted in the heads of wild type and claret after they have been given the double extraction described above. If the heads are extracted with Solvent B alone, they are white. It therefore appears that the treatment with Solvent A in some way interferes with the subsequent extraction by Solvent B. The pinkish color of the chitin and soft parts of the head may be evidence of retained red or brown eye pigment, but this is not certainly known, and in any case the amount left is extremely small.

RESULTS OF PIGMENT MEASUREMENTS

Pigment measurement data are contained in tables 6 to 10, inclusive. Table 10 summarizes in terms of mean values and percentage comparisons the data of tables 6 to 9 and will be useful for reference.

TABLE 6

The relative amount of red and brown pigment contained in extracts of intact whole heads of various stocks of Drosophila melanogaster. In this and the following three tables (table 6–9, inclusive) the numbers followed by an asterisk are the mean values for the pigment extracts used for comparative purposes in table 10.

LOT NO.	EXPER. NO.	STOCK	PHOTOMETRIC DENSITY	
			RED PIGMENT (SOLVENT A)	BROWN PIGMENT (SOLVENT B)
B-1 (37)	595	*bw*	0.020	
B-2 (56)	670–1	"	0.014	
			m=0.017*	
D/B-1	672–1	*v; bw*	0.0055	
(38)	672–2	"	0.0055	
			m=0.0055*	
	(BEADLE)	*v; bw*		0.0088
	"	"		0.0066
				m=0.0077*
	(BEADLE)	*cn bw*		0.0044
	"	"		c.0044
				m=0.0044*
A-2	61/	+Ore-R	0.848	0.1308
(29)	618	"	0.822	0.1382
	619	"	0.875	0.1352
	620	"	0.862	0.1163
	621	"	0.848	0.1249
			m=0.851	m=0.1291
A-3	663–1	"	0.862	0.1065
(50)	663–2	"	0.888	0.0969
	663–3	"	0.862	0.1024
	663–4	"	0.848	0.0996
			m=0.865	m=0.1013
			m=0.858*	m=0.1152*
C-1	606	*ca*	0.1264	0.0315
(12)	609	"	0.1235	0.0339
	610	"	0.1278	0.0339
(21)	614	"	0.1235	0.0339
			m=0.1253	m=0.0333*
C-2	622	"	0.1163	
(29)	624	"	0.1264	
	625	"	0.1337	
			m=0.1255	
			m=0.1254*	

TABLE 7

The relative amount of red pigment contained in extracts of the heads of vermilion, cinnabar, and their double recessive combination with claret.

LOT NO.	EXPER. NO.	STOCK	PHOTOMETRIC DENSITY (L) RED PIGMENT (SOLVENT A)
D-1	612	*v*	0.656 =0.656
(12)			
D-2	615	"	0.615
(29)	616	"	0.527
			m=0.571
D-3	664	"	0.706 =0.706
(16)			
			m=0.645*
D/C-1	631	*v; ca*	0.1110
(27)	632	"	0.1135
			m=0.1123
D/C-2	637	*v; ca*	0.1086
	638	"	0.1036
			m=0.1061
			m=0.1092*
E-2a	643	*cn*	0.783
(27)	644	"	0.809
	645	"	0.809
	646	"	0.809
			m=0.802
E-2b	650–1	*cn*	0.888
(28)	650–2	"	0.862
			m=0.875
E-3	665–1	*cn*	0.942
(19)	665–2	"	0.942
	665–3	"	0.942
			m=0.942
			m=0.873*
E/C-1	633	*cn; ca*	0.1575
(27)	634	"	0.1566
			m=0.1570
E/C-2a	676–1	*cn; ca*	0.1397
(38)	676–2	"	0.1397
			m=0.1397
			m=0.1484*

TABLE 8

The relative amount of brown pigment contained in extracts of the heads of the mutant brown and the double recessive, brown-claret.

LOT NO.	EXPER. NO.	STOCK	PHOTOMETRIC DENSITY (L) BROWN PIGMENT (SOLVENT B)	WEIGHT ♂♂ MG/100
B, B/C	724-1	*bw*	0.1135	23.4
(3)	724-2	*bw*	0.1121	
			m=0.1128*	
	724-3	*bw; ca*	0.0327	23.0
	724-4	*bw; ca*	0.0327	
			m=0.0327*	
A, B-1	723-5	*bw*	0.1135	23.0
(3)	723-6	*bw*	0.1135	
			m=0.1135*	
B/C-2	690-1	*bw; ca*	0.0327	23.5
(25)		*bw; ca*	0.0327	
			m=0.0327*	

TABLE 9

See text for discussion of data listed here.

LOT NO.	EXPER. NO.	STOCK	PHOTOMETRIC DENSITY RED PIGMENT (SOLVENT A)	PHOTOMETRIC DENSITY BROWN PIGMENT (SOLVENT B)	WEIGHT ♂♂ MG/100
A, C-1	691-1	+Ore-R	0.996	0.1308	23.7
(17)	691-3	"	0.996	0.1337	
			m=0.996*	m=0.1322*	
	691-2	*ca*	0.1457	0.0223	21.2
	691-4	"	0.1472	0.0188	
			m=0.1464	m=0.0205	
Corrected for weight difference between ♂♂			m=0.1636*	m=0.0229*	

Pigment Ratios:

$$\frac{ca}{+\text{Ore-R}} \qquad \frac{0.1636}{0.996}\times 100 = 16.4\% \qquad \frac{0.0229}{0.1322}\times 100 = 17.3\%$$

Control experiments are recorded in the upper section of table 6. Experiments Nos. 595 and 670-1 were independent determinations of the action of Solvent A on the heads of brown flies. In one experiment (595) the heads were extracted for 37 days, and in the other (670-1) for 56 days. The mean value of the two lots is 0.017, which corresponds to approximately 15.0

TABLE 10

Comparative summary of the data contained in tables 6–9.

STOCK	RED PIGMENT MEAN (L)	RED PIGMENT PERCENTAGE	BROWN PIGMENT MEAN (L)	BROWN PIGMENT PERCENTAGE
+Ore-R	0.858	100.0	0.115	100.0
ca	0.125	14.6	0.033	28.7
*+Ore-R	0.996	100.0	0.132	100.0
ca	0.164	16.5	0.023	17.4
v	0.645	100.0	—	—
v; ca	0.109	16.9	—	—
cn	0.873	100.0	—	—
cn; ca	0.148	17.0	—	—
bw	—	—	0.113	100.0
bw; ca	—	—	0.033	29.2
ca	—	—	0.033	29.2
+Ore-R	—	—	0.115	100.0
bw	—	—	0.113	98.2
ca	—	—	0.033	28.7
bw; ca	—	—	0.033	28.7
+Ore-R	0.858	100.0	—	—
v	0.645	75.2	—	—
cn	0.873	101.8	—	—

* Data shown in table 9, and discussed in text.

TABLE 11

Data showing the effect of temperature and age on extracts of the red pigment made with Solvent A. See text and figure 2.

DAYS	REFRIGERATOR 6°–8°C PHOTOMETRIC DENSITY (L)	REFRIGERATOR 6°–8°C (L) IN %	ROOM 20°–23°C PHOTOMETRIC DENSITY (L)	ROOM 20°–23°C (L) IN %	INCUBATOR 25°±0.5°C PHOTOMETRIC DENSITY (L)	INCUBATOR 25°±0.5°C (L) IN %
0	0.710	100.0	0.710	100.0	0.710	100.0
4	0.710	100.0	0.699	98.5	0.699	98.5
14	0.710	100.0	0.668	94.1	0.658	92.7
30	0.688	96.9	0.629	88.6	0.620	87.3
68	0.673	94.8	0.573	80.7	0.542	76.4

percent of the total value obtained when heads of the same mutant are extracted with Solvent B. On the basis of other evidence (see BEADLE and TATUM 1941) the particular allele of *bw* used here should block rather completely the formation of any water-soluble red pigment. Assuming this to

be true, the result of extracting brown heads with Solvent A may mean (1) that the water-insoluble brown pigment is to a certain extent (slowly) soluble in 30 percent acid ethyl alcohol, (2) that the eye pigment differentiated by the *bw* allele used here consists of two components one of which is soluble in Solvent A and the other not, (3) that under the conditions of the experiment, chemical changes occurred in the medium which rendered a portion of the otherwise insoluble brown pigment soluble in Solvent A.

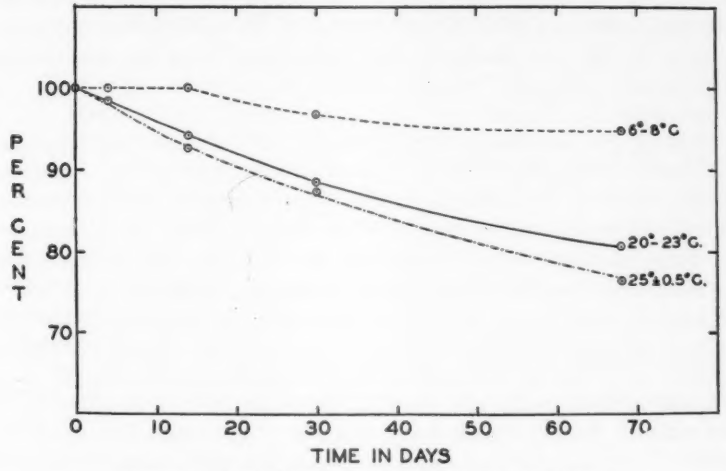


FIGURE 2.—Graphical representation of data given in table 11.

There probably are other alternatives, but the data on hand permit of no choice in explanations.

The other control experiments show the relative amount of colored material that can be extracted from the heads of stocks so constituted genetically as to block the formation of both the brown and the red pigment components. In the case of vermilion-brown extracted with Solvent A (672-1), the value obtained is about 0.5 percent of the maximum amount of colored material extractable from wild type or cinnabar with this solvent. On the other hand, vermilion-brown extracted with Solvent B gives a value which is approximately 6.7 percent of that obtained for wild type or brown with the same solvent. Cinnabar-brown extracted with Solvent B gives a lower value, corresponding to 3.8 percent of that from brown. This agrees with the observation that the eyes of the double recessive, cinnabar-brown, appear whiter than those of vermilion-brown. It is not known whether or not these basic values of colored material represent dissolved pigment derived from granules contained in the ommatidial cells, basement membrane, or from pigments unrelated to the eye.

The lower sections of table 6 give the data on wild type (+Oregon-R) and claret. The pigment relations between these two stocks given in terms of percent are shown in table 10. According to these data the claret mutant contains only 14 to 15 percent as much pigment extractable with Solvent B as does wild type.

Table 7 records measurements taken on stocks in which no brown pigment component is present, hence all extractions were made with Solvent A. The purpose here was to determine the action of the *ca* gene on the red pigment component alone. Comparisons in terms of percentage are given in table 10. The combination of claret with vermilion or cinnabar reduces the content of pigment to approximately 17 percent of that present in either alone.

Table 8 gives the data and table 10 the comparisons of the reciprocal arrangement wherein the effect of claret on the brown pigment component is demonstrated. The combination brown-claret contains about 29 percent as much pigment extractable with Solvent B as does brown alone. This value is almost identical with that obtained for the differential between wild type and claret with respect to the same pigment component and tends to encourage confidence in the validity of the double extraction method involving the use of Solvent A. However, in a final experiment (691, table 9) in which wild type and claret were raised in the same bottle, the sib males weighed, and the mean color values corrected for the weight difference of the males of the two stocks, there was no such agreement. In this experiment the relation between claret and wild type with respect to the red pigment is probably not significantly different from that previously noted. With respect to the brown component, however, the ratio is definitely different, claret in this experiment having only 17.3 percent as much pigment as compared to the 29 percent previously noted. This new value, 17.3 percent, is very similar to the values obtained for the differential action of the mutant gene claret on the red pigment component.

DISCUSSION

EPHRUSSI and CHEVAIS (1938) used the term *"l'autodifférenciation limitée"* to characterize the type of development a wild type optic disc undergoes when transplanted to a vermilion host. It should be clear that the so-called "claret effect" refers to this limited self-differentiation of the wild type optic disc. As shown by EPHRUSSI and CHEVAIS (1938) and by experiments reported in the first section of this paper, the wild type optic disc is dependent on sources other than its own tissues for enough v^+ hormone to develop its characteristic pigmentation. So far as is now known, v^+ hormone is concerned only in reactions leading to the formation of the

brown pigment component of Drosophila eye color; consequently, one might assume that the "lighter than wild type" appearance of wild type optic discs grown in claret and vermilion hosts is due to a deficiency for the normal complement of brown pigment. Although wild type optic discs can produce v^+ hormone (E. B. CLANCY 1940) when grown in vermilion hosts, the question arises as to whether or not such discs contain any brown pigment at all. To answer this, (+ in *v*) implants were extracted with Solvent A to remove the red pigment and compared under a binocular microscope with (+ in +) implants treated in the same way. Wild type discs from vermilion hosts show a definite residuum of brown pigment, but the amount present is less than that in (+ in +) implants. It was noted above that wild type implants recovered from vermilion (lacking v^+ and cn^+ hormones) and from claret (some v^+ and cn^+ hormones) hosts were indistinguishable by the ordinary method of observing such implants. There are at least two alternative explanations for this fact—either the quantity of brown pigment formed by virtue of the additional hormone supplied by the claret host is so small that a difference cannot be detected by the eye, or a definite concentration level of hormone in the host is necessary for the formation of additional brown pigment.

No account has thus far been taken of the possibility that the red pigment component of a wild type disc may be qualitatively or quantitatively affected by its development in hosts giving the "claret effect." Since the red pigment acts as a pH indicator and can be reversibly reduced to a colorless compound (see BEADLE and TATUM 1941), it is certainly possible that the particular reddish hue ("lighter than wild type") observed is conditioned by the pH and/or the oxidation-reduction potential of the medium in which the disc develops, or in which it is observed. Preliminary measurements (unpublished) of the red pigment from (+ in +) and (+ in *ca*) implants, however, indicate that there is no difference in the amount of red pigment they contain, but the data are inadequate for a proof of this point.

The claret gene is evidently concerned in reactions involved in the production (or destruction) of v^+ hormone. It is also concerned in the utilization of hormone in the production of pigment, since claret optic discs produce more v^+ hormone than they use in developing claret pigmentation (EPHRUSSI and BEADLE 1937). The latter fact makes it clear that the amount of v^+ hormone produced by claret eye tissue is not the limiting factor in determining the quantity of the brown pigment component in claret eye color. Since both pigment components are affected by the mutation $ca^+ \rightarrow ca$, the claret gene is evidently related in its action to the common step controlled by the white gene in the scheme of eye-color develop-

ment outlined by BEADLE and TATUM (1941). Any attempt to assign a single primary action to the claret gene in this scheme must relate reactions involved in the production of v^+ hormone to those controlled by the white gene.

LUERS and STUBBE (1940) have reported the results of tests for free ca^+ substance in the body fluid of the wild types of several species of Drosophila, including *D. melanogaster*. As test implants they used the optic discs of the double recessive, *w^e; ca*, of *melanogaster*. They found that the test implant developed a pigmentation only slightly darker than the control implant (one unit as measured on a Ridgway Color Chart). Eosin implants are three Ridgway units darker than eosin-claret. LUERS and STUBBE conclude that at the ages at which larvae are normally employed for these transplantations the wild type of all three species contains an insufficient amount of ca^+ substance [to permit development of the eosin phenotype?].

In view of the fact that the mutant gene claret limits the utilization of v^+ hormone to less than can be produced by its own tissues, a completely negative result in the case of these tests would have been more easily explained. The fact that some modification of the *w^e; ca* implant was apparently observed is not easily accounted for without further investigation.

SUMMARY

Two main problems are considered (1) the validity of evidence for the existence of ca^+ substance, and (2) the quantitative effect of the mutant gene claret on the eye-color pigments of *D. melanogaster*.

Results concerned with the first of these questions show that (1) the type of incomplete pigment differentiation exhibited by a wild type optic disc when grown in a claret host is nonspecific and occurs in other hosts known either to lack v^+ hormone or to contain it in reduced amount as compared to wild type; (2) differences in developmental age between the wild type implant and the claret host are not responsible for the partial pigment differentiation of the implant; (3) in comparative tests for v^+ hormone (release by Malpighian tubes and fat-bodies, and feeding tests of crushed whole pupae) claret gives lower values than wild type; (4) wild type optic discs grown in white-claret hosts (assumed to contain more v^+ hormone than claret) approach wild type pigmentation very closely.

In view of these results, the original argument for assuming the existence of ca^+ substance is no longer valid.

In connection with the second problem, a method for the extraction and measurement of Drosophila eye pigments is described along with the presentation of pigment measurements.

The data obtained in a series of measurements of eye-color pigment on wild type, claret, and double recessives of claret with vermilion, cinnabar,

and brown show that the mutant gene claret acts in such a way as to reduce the quantity of the red pigment to about 17 percent, and the brown pigment to about 28 percent of that present in wild type.

The relation of the claret gene to the scheme of eye-color development in Drosophila outlined by BEADLE and TATUM (1941) is discussed briefly.

ACKNOWLEDGMENT

I am indebted to DR. G. W. BEADLE, who suggested the problems, made many helpful suggestions, and furnished the unpublished results cited in table 1. Certain procedures used in making measurements of eye-color pigment were worked out by DR. G. W. BEADLE or jointly with DR. BEADLE and DR. E. L. TATUM. I am very grateful for their help. I am also indebted to my wife, ELEANOR B. CLANCY, for help in carrying out certain of the experiments.

LITERATURE CITED

BEADLE, G. W., 1937a Development of eye colors in Drosophila: Fat bodies and Malpighian tubes as sources of diffusible substances. Proc. Nat. Acad. Sci. **23**: 146–152.

1937b Development of eye colors in Drosophila: Fat bodies and Malpighian tubes in relation to diffusible substances. Genetics **22**: 587–611.

BEADLE, G. W., R. L. ANDERSON, and JANE MAXWELL, 1938 A comparison of the diffusible substances concerned with eye color development in Drosophila, Ephestia, and Habrobracon. Proc. Nat. Acad. Sci. **24**: 80–85.

BEADLE, G. W., and B. EPHRUSSI, 1936 The differentiation of eye pigments in Drosophila as studied by transplantation. Genetics **21**: 225–247.

1937 Development of eye colors in Drosophila: Diffusible substances and their interrelations. Genetics **22**: 76–86.

BEADLE, G. W., and L. W. LAW, 1938 Influence on eye color of feeding diffusible substances to *Drosophila melanogaster*. Proc. Soc. Exp. Biol. N. Y. **37**: 621–623.

BEADLE, G. W., and E. L. TATUM, 1941 Experimental control of development and differentiation: Genetic control of developmental reactions. Amer. Nat. **75**: 107–116.

BECKER, E., 1939 Über die Natur des Augenpigments von *Ephestia kühniella* und seinen Vergleich mit den Augenpigmenten anderer Insekten. Biol. Zbl. **59**: 597–627.

BUTENANDT, A., W. WEIDEL, and E. BECKER. 1940 Kynurenin als Augenpigment-bildung auslösendes Agens bei Insekten. Naturwissenschaften **28**: 63–64.

CLANCY, E. B., 1940 Production of eye color hormone by the eyes of *Drosophila melanogaster*. Biol. Bull. **78**: 217–225.

EPHRUSSI, B., and G. W. BEADLE, 1936a A technique of transplantation for Drosophila. Amer. Nat. **70**: 218–225.

1936b Development of eye colors in Drosophila: Studies of the mutant claret. J. Genet. **33**: 407–410.

1937 Developpement des couleurs des yeux chez la Drosophile: Influence des implants sur la couleur des yeux de l'hote. Bull. biol. **71**: 75–90.

EPHRUSSI, B., and S. CHEVAIS, 1937 Development of eye colors in Drosophila: Relation between pigmentation and release of the diffusible substances. Proc. Nat. Acad. Sci. **23**: 428–434.

1938 Developpement des couleurs des yeux chez la Drosophile: Relations entre production, utilisation et liberation des substances diffusibles. Bull. biol. **72**: 48–78.

GOTTSCHEWSKI, G., and C. C. TAN, 1938 The homology of the eye color genes in *Drosophila melanogaster* and *Drosophila pseudoobscura* as determined by transplantation. II. Genetics **23**: 211–238.

LUERS, H., and A. STUBBE, 1940 Der Gehalt an freiem ca^+-Stoff in der Drosophila-Lymphe. Z. i. A. V. **79**: 143–145.

MAINX, F., 1937 Analyse der Genwirkung durch Faktorenkombination. (Die Augenfarbe von *Drosophila melanogaster*.) Z. i. A. V. **73**: 470–471.

1938 Analyse der Genwirkung durch Faktorenkombination. Versuche mit den Augenfarbenfaktoren von *Drosophila melanogaster*. Z. i. A. V. **75**: 256–276.

MORGAN, T. H., C. B. BRIDGES and A. H. STURTEVANT, 1925 The genetics of Drosophila. Bibliogr. Genet. **2**: 1–262.

SCHULTZ, J., 1932 The developmental system affected by the genes for eye color in Drosophila. Proc. Sixth Int. Congress Genet. **2**: 178–179.

1935 Aspects of the relation between genes and development in Drosophila. Amer. Nat. **69**: 30–54.

TATUM, E. L., and G. W. BEADLE, 1938 Development of eye colors in Drosophila: Some properties of the hormones concerned. J. Gen. Physiol. **22**: 239–253.

1939 Effect of diet on eye-color development in *Drosophila melanogaster*. Biol. Bull. **77**: 415–422.

1940 Crystalline Drosophila eye-color hormone. Science **91**: 458.

TATUM, E. L., and A. J. HAAGEN-SMIT, 1941 Identification of Drosophila v^+ hormone of bacterial origin. J. Biol. Chem. **140**: 575–580.

MUTATION OF PHYTOMONAS STEWARTII BY X-RAY IRRADIATION[1]*

RALPH E. LINCOLN AND JOHN W. GOWEN
Iowa State College, Ames, Iowa

Received January 24, 1942

VARIATION in cultural characteristics, viability, and motility of bacteria has been recognized almost from the beginning of their study in pure culture. Because of the influence of the Koch school, which emphasized cultural purity, early bacteriologists were prone to attribute changes in cultures to contamination. Thus, many of the earlier observations of bacterial variation probably were discounted or misinterpreted. Variations associated with virulence and antigenic types, however, have become so important, particularly from the medical standpoint, that in certain forms such as the pneumococci many variant types have been described, and many of the cultural conditions necessary for the full growth of the particular variant types have been made known. Lacking critical experiments to prove their cause and nature, these variant strains ordinarily have been referred to as dissociants, variants, and saltants arising by some process vaguely defined as "dissociation," and, with the exception of certain workers, the terms "mutant" and "mutation" as ordinarily applied to variation in higher organisms such as maize, Drosophila, and man have been avoided.

Superficially, if we neglect the differences in reproductive capacity of the mutants, the observed changes and the experimental conditions surrounding the changes suggest that variation is caused by the environment acting on the organism rather than by spontaneous changes within the organism. In a proper environment a parent culture may be replaced by one of its variants. In some cases the environment necessary to facilitate the appearance of these variants is known and may be controlled to some degree. In light of such facts it is not surprising that environmental differences have been interpreted as causative in initiating variation rather than correlative and that the mutation concept is often regarded as unnecessary to account for the variants observed.

The problem of the origin of variation is of particular importance to the bacteriologists' concepts of species and the geneticists' outlook on the mech-

[1] Journal paper J-994 of the IOWA AGRICULTURAL EXPERIMENT STATION, Genetics Section, Projects 404–573. Part of this work was done during tenure by the senior author of a National Research Council Fellowship in the Natural Sciences at CORNELL UNIVERSITY, Department of Plant Pathology, Ithaca, N. Y. The senior author wishes to express his appreciation to DR. W. H. BURKHOLDER for providing facilities for work and helpful suggestions and to MR. T. H. SPROSTON for photographic assistance and equipment.

* The cost of the accompanying halftone illustrations has been borne by the Galton and Mendel Memorial Fund.

anism and permanence of inheritance. To analyze this problem, we need to know the quantitative aspects of the behavior of the parent and mutant bacterial forms under a variety of environments. Then an analogy may be drawn to the same quantitative reaction as is expressed by specific genes under like conditions in higher forms where mutation is the mechanism considered responsible for variation. Penetrating irradiation offers one means of studying this problem, the success of which has been demonstrated in analyzing the fundamental aspects of variation and heredity in multicellular organisms.

Little use has been made of irradiation in modifying the hereditary constitution of the bacterial cell. Experiments with X-rays on unicellular organims have dealt largely with inactivation or killing effects rather than with mutation. Either the mutations observed were few or they were regarded as quantitatively unimportant. Those that have been reported have dealt with immunological and growth phenomena, as in the report of LANGE and FRAENKEL (1923) on the reduced virulence of a human strain of tubercle bacillus when inoculated into guinea pigs, or have been qualitative, as in the report of Haberman and Ellsworth (1940) in which they show that X-rays increased the amount of "dissociation" in *Staphylococcus aureus* and *Serratia marcescens*.

This paper presents a study of variation in the unicellular organism *Phytomonas stewartii* (E.F.S.) Bergey, et al., as it occurs spontaneously on culture media and as it occurs on the same media after the organism has been exposed to X-ray irradiation. *Ph. stewartii* is a non-motile bacterium of medium length. Its natural host is maize, in which it produces a vascular wilt disease. The organism may assume epiphytotic virulence for maize in certain areas when local conditions have been favorable. On solid media each bacterial strain has a definite growth configuration as expressed in colony type and size. Colony color is normally yellow. A quantitative estimate of the type and rate of occurrence of hereditary variation in these characters both spontaneously and during exposure to X-ray irradiation is presented below.

METHODS

Bacteria were irradiated with X-rays from a gas-type tube of the general design of WYCKOFF and LAGSDIN (1930). An exact description of this particular tube has been presented by PINNEY (1939). The targets were pure metals—copper or silver. The majority of the experiments were with X-rays from the copper target. The X-rays were filtered heavily by a window of palladium for the silver target and nickel for the copper target, the thickness of the window being adjusted to absorb about 50 percent of the irradiation. The current through the tube during irradiation was held constant at 12.5 milli-amperes. Beam intensity at the time of irradiation was

measured either by a small ionization chamber or a Victoreen dosimeter. The ionization chamber was designed by PINNEY following the general plan of TAYLOR and SINGER (1930). The Victoreen dosimeter was checked frequently with this ionization chamber.[2]

The average effective wave length of the X-rays obtained from irradiation of the silver and copper targets under the above conditions was determined by absorption experiments through successive sheets of aluminum 13×10^{-4} and 72×10^{-4} cm in thickness. Results of these tests showed the average effective wave length of the silver irradiations to be 0.7Å (17.6 kv) and of the copper to be 1.5Å (8.2 kv).

Bacteria were irradiated in straight-walled dishes 18 mm in diameter and 5 mm deep made from a block of paraffin. Into each dish 0.75 ml of 14–18 hour nutrient broth culture of *Ph. stewartii* was introduced, and the dish was sealed with cellophane to prevent air contamination. The number of living bacterial cells after treatment was estimated from the known inactivation rate established under like conditions in previous experiments. Employing this estimate, a dilution was made that allowed most colonies on a plate to develop individually. The number of colonies desired varied with the characteristic size and consistency of the colony of the strain treated. One drop of broth of the desired dilution was placed on the surface of the hardened nutrient dextrose agar plate and smeared evenly over the entire surface of the plate by means of an L-shaped glass rod. Plates were incubated at room temperatures for 48 hours, then observed at 16× magnification for colony changes from the normal as regards color, surface, or size. The observed variants were suspended in broth and plated again to ascertain whether or not the variations were genetic. Amount and kind of variation present before treatment was determined in all cases. All dilutions and smearing of the bacteria on the plates were completed in less than 20 minutes after treatment. Since the maximum time of irradiation was 25 minutes, treatment and plating was completed in a period much less than the generation time of *Ph. stewartii*, which is greater than 1.5 hours in the media used.

Since the bacteria were suspended in broth, special techniques were necessary to measure the X-ray dosage. Since these problems will be discussed in another paper, we shall limit the data herein to the following: the frequency of variants under natural conditions, the changes in this frequency brought about by irradiation with X-rays, a description of the frequency and rates at which the different types of variants appear under the circumstances, and the virulence and stability of the different variants.

Experiments with the effect of X-rays of different wave lengths on the

[2] The authors are deeply indebted to the FULLER FUND and the INTERNATIONAL CANCER RESEARCH FOUNDATION for cooperating in these investigations by supplying the above equipment.

inactivation of these bacteria showed that survival during irradiation follows the simple exponential function in which the survival is inversely proportional to the dosage. The general equation for this relationship is

$$\text{survival ratio} = e^{-ar}$$

where a is a constant and r is the roentgens to which the bacteria are exposed. The fact that inactivation follows this expression suggests that one unit of energy absorbed by the bacterial cell is sufficient to kill it if the absorption takes place in the vital region. This expression holds under a variety of conditions and thus may be used as an accurate measure of roentgen energy absorbed by the bacteria under conditions of treatment where it is difficult to measure the absorbed energy directly. For this purpose the expression above takes the following form:

$$r = \frac{\log \text{survival}}{a \log e}.$$

Since a and log e are constants, the log of the survival ratio is a sufficiently accurate measure of dosage. This measure is the one used in the text.

Throughout this work it was assumed that most colonies start from a single bacterium and that any change in the heredity of the individual cell that affects colony color, type, or size may be observed after the cell is placed on agar and allowed to develop into a colony. The assumption as to the single-cell origin of these colonies is not appreciably "less real than ideal" DUGGAR (1936) and has experimental verification in the work of MCNEW (1938) and LINCOLN and GOWEN (in press). By direct observation of seeded poured plates MCNEW observed that cells of *Ph. stewartii* were distributed singly throughout the agar in all but about 1 percent of the cases. LINCOLN and GOWEN, by studying the progeny of mixtures of yellow and white or rough and smooth strains of *Ph. stewartii*, showed that the bacteria in these mixed cultures had an equally low rate of sticking together. Furthermore, X-ray experiments on this species showed that the bacteria were inactivated in a manner comparable to individual particles as contrasted to the curve characteristic of two or more attached particles. The observation that, except for the sectored colony types, most mutant colonies are pure and stable from the time of origin is a further indication of the single-cell origin of most colonies.

BACTERIAL STRAINS USED AND KIND OF VARIATION OBSERVED

Two strains of *Ph. stewartii* were used for these experiments—S15 (400), a rough colony type, and A14 (500), a smooth colony type.

Classified in table 1 is the variation observed in S15 stock. The normal colony type of S15 (Plate I, A) is a small, compact, slightly rough, dark

yellow colony. This type has arbitrarily been called RI and other rough types designated by RII—RVI (Plate I, B, C, D, E). These types differ principally in the degree of roughness and in the elevation of the colony. Smooth variants were observed, but no attempt was made to classify these into a smaller unit, because any particular smooth variant occurred less frequently than did any particular rough variant. Smooth types varied

TABLE 1

Occurrence of mutation in Phytomonas stewartii under natural conditions and after X-ray treatment.

CHARACTERS OBSERVED STOCK S15 (400)	NUMBER OF MUTANTS OBSERVED		
PARENT TYPE IS DARK YELLOW IN COLOR, ROUGH I IN COLONY TYPE	SPONTANEOUS (BROTH)*	AFTER IRRADIATION	
		17.6 kv	8.2 kv
Mutational changes involving only one character			
Color change from dark yellow to:			
Pale yellow	29	11	72
White	16	3	22
Colony type change from Rough I to:			
Rough II	8	14	38
Rough III	2	5	4
Rough IV	2	2	4
Rough V	0	1	3
Rough VI	0	0	1
Smooth type	16	4	4
Colony size change from normal to:			
Small	1	18	10
Mutational changes involving more than one character			
Pale yellow—Rough II	1	2	2
Pale yellow—Rough III	0	1	1
Pale yellow—Rough IV	0	4	6
White—Rough II	1	0	12
White—Smooth	0	1	0
Yellow—Smooth—Mucoid	2	0	1
Small colony—Pale yellow—Rough I	0	6	2
Small colony—Pale yellow—Rough II	1	14	5
Small colony—Pale yellow—Rough III	0	2	1
Small colony—White—Rough I	1	5	1
Unstable variants	0	11	4
Sectors	12	36	80
Total	92	140	272
Colonies observed	1,524,000	9,360	386,000

* Minimum number. See text.

from homogeneously granular to translucent (Plate I, F, G, H, I, J). The predominant smooth type was an intermediate form developing a chromogenic center within a translucent outer portion. The pattern of the chromogenic material was relatively constant in a given environment but between environments was subject to change more than the other characters described. Mucoid smooth forms from the non-mucoid S15 rough type were observed. Small colony types (Plate I, K) occurred, particularly after irradiation. In stock S15 the change of colony color from dark yellow to pale yellow or to white represented another frequent change. Sectored colonies of the various types also were observed (Plate I, L, M, N), as were other colonies classified as unstable because stocks of such types could not be purified for the variant characteristics. The change of colony color from dark yellow to pale yellow and to white was observed.

Strain A14 is characterized by large, spreading, smooth, mucoid diffusely grayish-colored colonies (Plate II, A). Variation within this strain has been studied less than in the S15 strain because the colonies of A14 are less favorable material for variation study than are those of S15 due to their tendency to coalesce and their larger size.

The variations observed in A14 are classified in table 2. Two groups of smooth variants were observed frequently. The first group (Plate II, B, C, D, E, F) was similar to the parent in size and mucoidness of colony, but the second group (Plate II, G, H, I, J, K, L) was composed of dry mucoid, small-colonied smooth types consisting entirely of highly chromogenic material. In this latter group a single mutation from mucoid to non-mucoid was observed. A rough-mucoid type (Plate II, K) appeared to be the mutant commonly observed to occur during susceptible host passage (LINCOLN 1940) and appeared completely rough for the first two days' growth, then formed a smooth halo around the rough center. Completely rough mutants of two types were observed (Plate II, M, N). A rather frequently ob-

---

PLATE I. Mutants observed in the S15 (400) series.

A.—Parent type. Colony highly chromogenic, dark yellow in color, and of RI type surface appearance.

B, C, D, E.—Other rough types of colonies (RII, RIII, RIV, and RV, respectively) observed as mutations from A. All are composed entirely of chromogenic material.

F, G, H, I, J.—Smooth types of colonies observed as mutations from A. F is entirely chromogenic, while J is entirely non-chromogenic. G, H, and I are intermediate forms having a center of chromogenic material surrounded by non-chromogenic growth.

K.—Small colonies in which are mixed two normal sized colonies to show comparative size. ×1½. Four day old colonies.

L.—Sector. RIV sector arising in RI colony. ×5.

M and N.—Sectors. Bursts typical of growths occurring from the unstable type of colonies. ×2. Three day old colonies.

Colonies A–J magnified 4 diameters and photographed by reflected light after 48 hours' growth on nutrient dextrose agar.

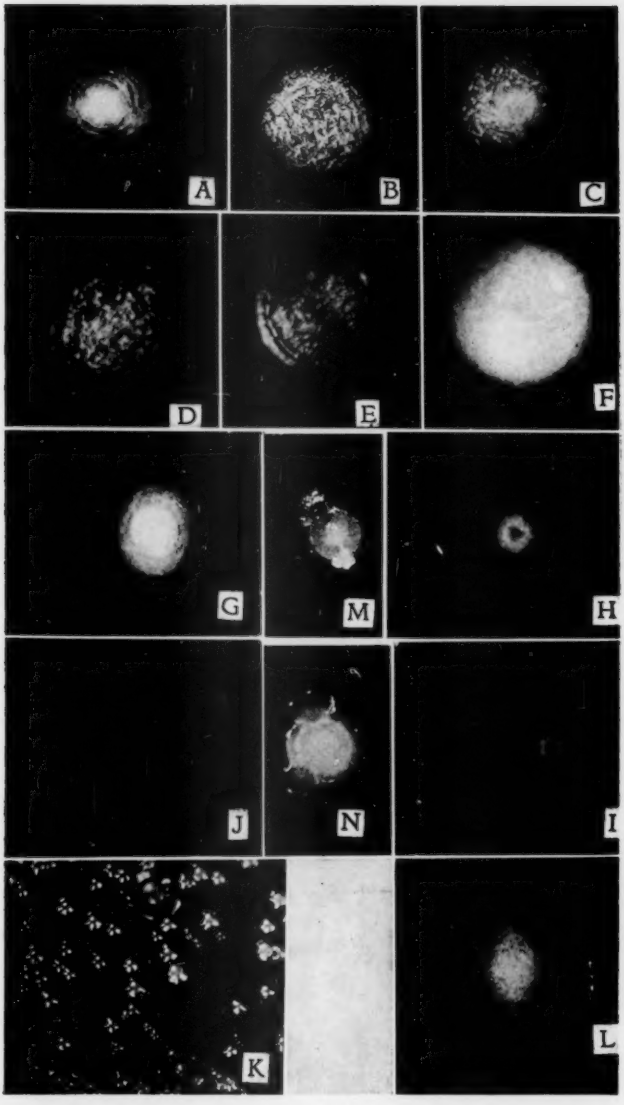


PLATE I

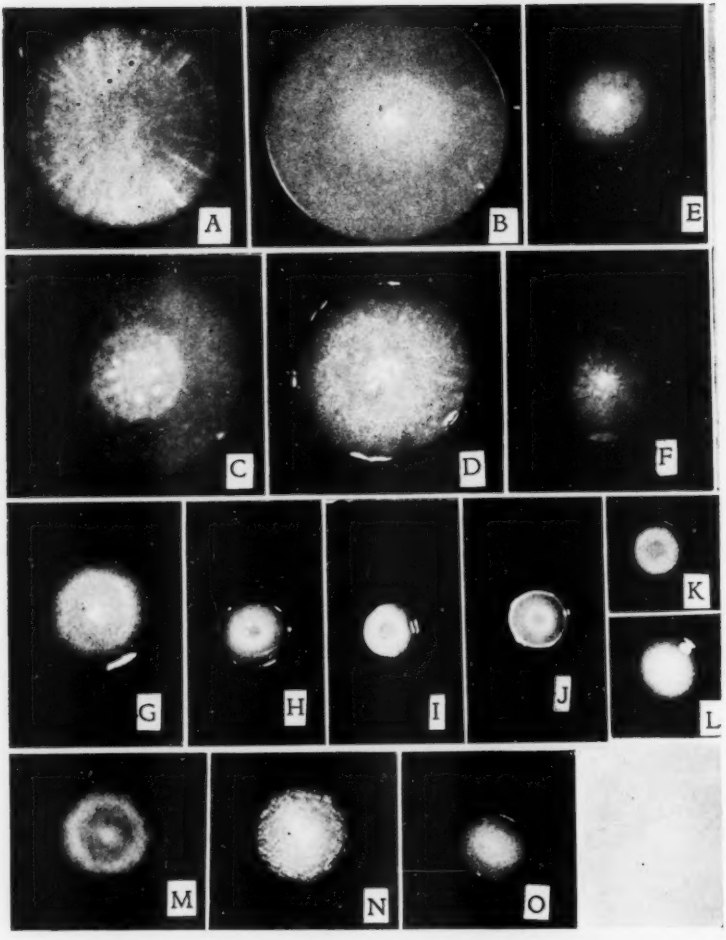


PLATE II

TABLE 2

Occurrence of mutation in Phytomonas stewartii under natural conditions and after X-ray treatment. (17.6 kv).

CHARACTERS OBSERVED STOCK A14 (500)	NUMBER OF MUTANTS OBSERVED	
NORMAL IS PALE YELLOW COLOR, SMOOTH COLONY WITH SLIGHTLY STRIATED CENTER, VERY MUCOID.	SPONTANEOUS (BROTH)*	AFTER IRRADIATION 17.6 KV
Mutational changes involving only one character.		
Color change from pale yellow to:		
Dark yellow	1	2
White	—	1
Colony type change from large smooth colony with undifferentiated center to:		
Large smooth, differentiated center	3	3
Small smooth, compact and dry	8	9
Rough center, mucoid smooth edge	2	1
Rough	—	2
Colony size change from normal to a small watery scant type of growth	4	3
Mutational change involving more than one character, from parental to:		
Dark yellow—Rough mucoid	—	1
White—Small dry smooth	—	1
Small smooth—Non-mucoid smooth	—	1
Unstable variant	—	1
Total	18	25
Colonies observed	156,000	24,378

* Minimum number. See text.

PLATE II. Mutants observed in the A14 (500) series.

A.—Parent type. Sticky-mucoid, large-colony type having slight internal striations of highly chromogenic material.

B, C, D, E, F.—Sticky-mucoid, large-colony, smooth types having a center differentiation of highly chromogenic material.

G, H, I, J, K, L.—Dry-mucoid, small-colony, smooth types consisting entirely of highly chromogenic material.

M.—Intermediate (RM) type. Center chromogenic material is rough type of dry-mucoid consistency (like G to O); outer, halo-like material is non-chromogenic and is of sticky-mucoid consistency (like A to F).

N and O.—Rough types consisting entirely of highly chromogenic material of dry-mucoid consistency.

Colonies of figures C, F, and G were observed only from spontaneous variation; other figures were observed after X-ray treatment. Photographed by reflected light. Colonies 48-hours old on nutrient dextrose agar. Magnification ×3.

served small, translucent colony type proved to be the only mutant difficult to maintain on culture media. In addition to these morphological mutations the changes from pale yellow to dark yellow and to white were observed.

RATE OF MUTATION OF COLONY CHARACTERISTICS

Summarized in table 1 are the data from numerous experiments to determine the spontaneous mutation rate of colony color, surface, and size characteristics. For this material, bacteria from a single colony were used to inoculate a tube of nutrient broth. After 18–20 hours' growth at 27°C the cultures were diluted, surface-plated on agar, and grown for 48 hours. Colonies then were examined for variants, and a minimum mutation rate was calculated based on the tube as a unit. Repeated observation of the same variant in a single tube was considered to indicate merely its growth after origin and not its repeated origin. In these experiments the minimum mutation rate ranged from zero to 137 mutants per million cells observed, the average being 46.5. Most uncontrolled factors, such as mutant cell lethality, lower viability, a possible segregation mechanism, and random loss of mutants in sampling, tend to keep the observed mutation rate at a minimum. The average and the range of the minimum mutation rates were of the same order of magnitude as those observed for mutation of specific characters in higher organisms where observed variants are ordinarily attributed to mutation of specific genes.

The different types of mutation with S15 stock are separated in table 1 into three groups—those arising spontaneously and those treated with X-rays of 17.6 kv and 8.2 kv. X-ray mutants are not different qualitatively from those that appear spontaneously, since most types of mutations appear in each group. The difference in the occurrence of mutation under natural conditions and X-irradiation lies in the frequency of mutations rather than kind of mutation produced. An example of this fact is found in table 1. Spontaneous mutations were found in 92 bacteria out of 1,524,000 colonies observed. Of these 92 bacteria several varied in two or more characteristics making a total mutation of 109 different characters. For the bacteria treated with 8.2 kv there were 272 mutated bacteria out of 385,919. Two or more mutations occurred in several individuals of this group, the total being 310 mutations. The surviving cells observed for the different experiments from which these data were drawn ranged from 252 to 37,000,000 per 100,000,000 in the initial culture. If we choose the point where 8,000 organisms survived out of the initial 100,000,000, the rate of mutational change was 4.2 per 1,000 colonies. This point corresponds to roughly 38,000 roentgens incident to the bacteria. The average (and distinctly tentative) rate of visible mutation per roentgen per bacteria there-

fore corresponds to 1.1×10^{-7}. As may be noted from table 1, three major characteristics were emphasized in determining the frequency of these different changes. Colony color may be dark yellow, pale yellow, or white. Colony surface may be smooth or rough, the degree of surface roughness being dependent upon the particular mutational change. At least five different classifiable and subsequently reproducible mutations affecting surface roughness are found. Colony size may vary from a very small colony to one of two or three times the size of the parent type. The average mutation rate per locus studied therefore would be less than 1.1×10^{-7} by at least a factor of three to account for the three different characters utilized in determining these frequencies. In this paper the rate of change of these bacteria under X-rays will be considered as approximately 3.7×10^{-8} per character per roentgen.

In their work on the effect of X-rays of 6.0 kv, 8.3 kv, and 17.4 kv, FRYER and GOWEN (1941) found 49 visible mutations in 168,945 gametes treated with roentgen dosages between 2,475 and 7,250 incident to the *Drosophila melanogaster* sperm. The rate of change per gene per roentgen was calculated as 7.0×10^{-8}. Besides these verifiable mutations following X-ray treatment, F_1 individuals were observed with one of the recessive characters. The occurrence of these individuals indicate either a mutation of the irradiated wild type allele to the recessive type or a deficiency in that region. Tests for the genic nature of these later changes could not be made because of sterility or early death of the mutant. The total of these changes plus the verified gene mutations was 123, or the rate of observed mutational change was 1.7×10^{-7} per gene per roentgen. This rate of change may be high as judged by the rates of mutation obtained by other workers. For the loci of forked, miniature, and eosin the data collected by TIMOFEEFF-RESSOVSKY (1933) indicate rates of 6.1×10^{-8}, 2.4×10^{-8}, and 2.6×10^{-8}. For sex chromosome genes MOORE, cited by JOHNSTON and WINCHESTER (1934), found 48 mutations in 116,200 gametes following a dosage of 3,975 roentgens or a mutational rate of 1.0×10^{-7}. The rates of mutation observed in this paper for *Ph. stewartii* are therefore easily within the range of those that one might expect for the genes of higher organisms.

One possible criticism against comparing the spontaneous and X-ray mutations should perhaps be discussed. Since the X-rays kill off or inactivate the bacteria from 100,000,000 down to 1,000, it might be argued that the killing occurred chiefly in the parental form, thus leaving any spontaneous mutants to show a spurious increase in number. This criticism is easily put to experimental test where the inactivation rates of mutant and parental forms are determined for like roentgen dosages. We have made several such tests on mutants of this bacterium and have always found the same inactivation rates for the different mutants and their parents. This

furnishes strong evidence that we are dealing with true genes and gene mutations.

In the data of table 1 the distribution of the different types of mutations as they arose spontaneously contrasted with the same types as they arose through X-rays show a highly significant difference, the χ^2 value being 70 for 7 degrees of freedom. The percentage relative frequency of different mutations as reported in table 1 is shown in table 3.

TABLE 3

Percentage frequency of spontaneous mutations and mutations following X-ray treatment.

	SPONTANEOUS	X-RAY	P
Dark yellow to pale yellow	32	26	.15
Dark yellow to white	18	9	.01
Rough I to other roughs	15	24	.05
Rough I to smooth	18	2	.01
Normal colony size to small	3	13	.01
Stable to unstable forms	0	3	.01
Uniform colonies to sectored	12	23	.01
Normal to mucoid	2	1	.02

The mutation from dark yellow to pale yellow (table 3) occurs with nearly equal frequency in the spontaneous and in the X-ray group. However, 18 percent of the spontaneous in contrast to nine percent of the X-ray mutations are from dark yellow to white colonies, a difference that is highly significant. Significant differences in the percentage frequency of the mutations in the spontaneous and in the X-ray group are found for all other mutations observed. The mutations from Rough I to smooth colony types and from normal to mucoid occur at greater frequency spontaneously than during X-ray treatment. In the other cases the mutations are more frequent in the X-ray group than in the spontaneous group. The fact that no unstable mutants were found in the 109 mutations of the spontaneous class whereas 15 were found in the X-ray group of 310 mutations is of interest in view of a similar comparison observed in Drosophila mutation. Variegated types in Drosophila are more frequent in X-ray material than in untreated material.

Necessary differences in technique could explain some of the observed difference between spontaneous and X-rayed bacteria. Through necessity the spontaneous types represent the accumulated changes over a period of 18 or more hours' growth. At any one of these cell divisions a mutation could occur. If reproducing at the same growth rate as the type form, the mutation would increase geometrically in numbers but remain in the same proportion to the type cells. If the mutation has a faster or a slower growth

rate or is unstable, reverting to type, etc., the proportion would not represent the original rate of change. Such special conditions could create bias toward the type form in the spontaneous data not found in the X-rayed material.

The question arises as to whether or not similar differences in mutation frequency exist in material treated by two types of X-rays. Data on this point are presented in table 4.

TABLE 4

Percentage frequency of mutations following treatment with X-rays of 8.2 kv and 17.6 kv.

	8.2 KV	17.6 KV	P
Dark yellow to pale yellow	29	21	.06
Dark yellow to white	11	5	.01
Rough I to other roughs	25	24	.81
Rough I to smooth	1.6	2.6	.48
Normal size colony to small	6	24	.01
Stable to unstable forms	1	6	.01
Uniform colonies to sectorial	26	19	.08
Normal to mucoid	1	0	.48

Most of the mutation frequencies found in the material irradiated by X-rays of 8.2 kv are similar to those found when X-rays of 17.6 kv were used. There are three exceptions—namely, X-rays having the greater energy induced changes to the small colony type and to unstable forms with a greater frequency than the X-rays of the lower energy, while the change from dark yellow to white colony color occurred with lowered frequency.

A similar comparison has been made between the spontaneous mutations and the X-ray mutations in the A14 stock as tabulated in table 2. The numbers of mutations observed in these experiments are scarcely sufficient to establish differences in rates. This is borne out by the fact that tests of significance for the spontaneous changes and X-ray changes show that the proportion of different types of mutants is essentially the same in each condition.

The results thus far discussed show that besides greatly increasing the frequency with which most mutations occur, X-rays also may tend to increase the proportion of certain types of variants. The significant differences between mutation under natural and X-ray conditions do not obscure the fact that there is a definite uniformity in mutation under the two conditions. This similarity supports the view that the mechanism by which the change is accomplished is similar in the two cases.

MULTIPLE MUTATIONS IN S15 STOCK

Colony color, surface appearance, and size are characteristics that have been observed to mutate. Each characteristic generally mutates singly, the occurrence of individuals showing two or more simultaneous changes being a rare event. In the spontaneous mutant class the total number of mutant colonies observed, after omitting the unstable and sectored groups, included 74 with a single mutation, five with two mutations, and one with three mutations—a total of 80 mutant colonies or a total of 87 character changes in 1,523,988 bacteria. If the production of two mutations in the same individual were independent, the probability of mutation of two or more characters would be obtained from the exponential equation

$$ne^{-m}\left(1 + m + \frac{m^2}{1.2} + \frac{m^3}{1.2.3} \cdots\right)$$

in which m is the mean mutation rate.

The rate of multiple mutation is much greater than is expected from this probability. Actually, five colonies with two mutations and one with three mutations were observed. Comparably high rates of multiple mutation have been observed in the X-ray classes. The frequency of these different types of mutations is found in table 5.

TABLE 5

Frequency of bacteria showing all parental type characteristics, one mutant character, two mutant characters, and three mutant characters.

VARIANT CHARACTER PER BACTERIUM	ACTUAL			EXPECTED*		
	SPONTANEOUS	8.2 KV	17.6 KV	SPONTANEOUS	8.2 KV	17.6 KV
0	1523908	385727	9220	1523907.9980	385727.0385	9220.4634
1	74	158	58	79.9960	188.9148	92.0760
2	5	25	19	.0021	.0462	.4598
3	1	6	16	.0000	.0000	.0015

* The average total X-ray dosage for the 17.6 kv is considerably higher than that for the 8.2 kv group. This fact is reflected in the proportionately greater number of variants observed.

It has been seen that multiple mutations occur at a frequency considerably higher than that expected by chance. The question may arise as to whether or not this increased frequency is alike in each of the three groups —spontaneous, 8.2 kv, and 17.6 kv irradiation. The comparison of the mutation frequencies of spontaneous and 8.2 kv mutation gives a probability of similarity between them of 0.05. The comparison of the spontaneous group with the 17.6 kv group gives a probability of considerably less than

one in 1,000 that they are the same in the distribution of their mutants. A similar comparison of the 8.2 kv and 17.6 kv X-ray groups shows these groups also to be distinctly dissimilar. This dissimilarity occurs principally in the increase of double and triple mutations within individual bacteria in the higher voltage group. Since a greater amount of energy is available to the photoelectron of high voltage as compared with those of low voltage, it would seem to follow that the absorption of such high energies in the bacteria favor the mutation of two or more genes within the organism. It is possible that in the wave lengths used in these studies there is a region comparable to that between the X-rays and the ultra-violet, as demonstrated in Drosophila, where the energy of X-rays is sufficient to induce large numbers of translocations and deficiencies as well as intra-molecular changes, while in contrast, ultra-violet irradiation is sufficient to induce only intra-molecular changes.

To explain the higher frequencies of double and triple mutations observed in both the spontaneous and X-ray groups it is necessary to invoke other hypotheses than those already mentioned. Three such hypotheses suggest themselves.

(1) A single mutation in hereditary material may change the expression of two or more phenotypic characters.

(2) In the mechanics of the mutation process a single event capable of producing a mutation may carry over to two or three genes instead of just one.

(3) A mutation when observed causes the observer unconsciously to examine that colony with extreme care, and more changes may be noted than otherwise would be the case.

The discussion of these hypotheses is complicated by the high frequency of the 0 class.

In view of the many known cases of multiple effects of genes in higher organisms, the first hypothesis would appear to be a reasonable explanation. In Drosophila and other organisms, many genes are known that affect the characteristics of two or more organs. It long has been recognized in bacteria that changes in virulence, antigenic properties, and other characteristics often accompany or are associated with the change from rough to smooth colony type. Such associated changes could be due to the multiple effects of a single gene. This explanation is not entirely satisfying, however, since in our experiments each of the characters studied ordinarily arises as a single change.

The second hypothesis has possibilities of two or more subsidiary hypotheses. In table 5 are listed the expected frequencies of no, one, two, and three mutations per bacterium on the assumption, expressed earlier in this paper, that the appearance of one mutation is independent of another in

each of the groups. It is obvious that the corresponding expected curves do not fit the actual data in any of the three groups—spontaneous, 8.2 kv, and 17.6 kv X-ray. In each case the proportion of double and triple mutations is too large. This observation would appeal to those who interpret mutational effects of X-rays as dependent upon the surrounding medium being first activated, the excitation causing lability of the genes enveloped by the activated medium. Should such an action take place, it would be fairly easy to account for the higher frequency of two and three character changes. On the other hand, were this true, the X-ray treatments would be expected to show proportionately higher numbers of multiple mutations than the spontaneous group, since X-rays of the order used have ample electronic energy to cause such chain reactions. Just the opposite result was observed, however. In the spontaneous data five double mutations were observed (not including triple mutations) where the expectation is only 0.0021, or the rate is 2,380 times expectation. With X-rays of 8.2 kv there are 25 doubles when 0.046 were expected or the rate is 534 times expected. For the 17.6 kv X-rays there are 19 double variants where 0.46 are expected, a rate 41 times expectation. The spontaneous rate is 58 times greater than the 17.6 kv X-ray rate, thus militating against this interpretation.

But this argument does not entirely settle the question, for the data may be interpreted from a different viewpoint, a viewpoint which is no less real than that presented above. Mutation in a bacterial cell could be due to two or more independent causes—that is, cells may be sensitized by one agent so that another agent is then able to produce a mutation in greater frequency than in an unsensitized cell. The course of such chain causes leading to mutations could be illustrated by X-ray effects. It might be assumed that in order to mutate at all the cell had to absorb a photoelectron and then become sensitive. But whether or not the cell would mutate again in any of its genes would depend on the direction of the ion path traced by the photoelectron. For the purposes of this problem we should look only to the cells which had mutated at least once and then compare the actual rates for two or more mutations with those expected on chance. Data for the spontaneous group show 80 cells which contained at least one mutation. The mean frequency for further mutations is 7/80 or 8.75 percent. The expected chance distribution for these cells then is 73.4 cells with one, 6.3 with two, and 0.3 with three mutations. This distribution agrees very well with the observed. It would seem that once the untreated cell is prepared for mutation, repeated mutation could be attributed to some agency acting by chance.

The X-ray data, when analyzed in like manner, go counter to this interpretation. The binomial equation attempts to compensate for the large

numbers of triple mutations by too low a number of single and too a great a number of double mutations. The expected numbers even then fail to reach the observed frequency of triple mutations. This evidence would favor the view that the agency making for the original mutations in itself tends to increase further mutations as might be true if two or more mutations came from a single ion tract. This view is further borne out by the higher frequency of triple mutations in the 17.6 kv irradiations where higher roentgen exposures were used and the tract of the photoelectrons is longer.

The data give only an inconclusive analysis of this second hypothesis. However, they do suggest one other point. The mechanism of mutation for the spontaneous group appears different from that of the X-ray group. Since radiant energy was used in the X-ray group, this suggests that radiant energy—that is, in the form of cosmic rays—is not responsible for the mutation in the spontaneous mutation group, a conclusion which would agree with the present viewpoint on mutations in higher animals.

The third hypothesis is difficult to evaluate. All colonies were carefully examined and any bias certainly is not intentional.

MUTATION IN A14 STOCK

The distribution of the different mutant types found in A14 stock is presented in table 2. This stock is characterized by large, spreading, smooth, mucoid, diffusely gray-colored colonies. In contrast to S15, A14 is pale yellow, smooth colony type. Table 2 shows that A14 may mutate from this pale yellow type to either the dark yellow or the white. It also may mutate from the smooth to the rough forms. In fact, the most frequent variants are either a rough mucoid type, in which the colony appears rough for about the first two days of growth and then forms a smooth halo around this rough center, or small, darker-yellow smooth mutants that form a dry compact mucoid colony. A single mutation from the mucoid type to the non-mucoid was observed.

It may be of interest to note that not all the variants are of equal viability. A small transparent colony type that was isolated from A14 proved to be difficult to keep alive on culture media. This forms another point of similarity between the variants in the bacteria and those of Drosophila, for many of the Drosophila variants are not so viable as the parent types.

The results with S15 and A14 stocks make it evident that *Ph. stewartii* can change from dark yellow to pale yellow or to white or from pale yellow to either dark yellow or white. It may also change from rough to smooth or from smooth to rough, from non-mucoid to a mucoid growth or in the opposite direction. The variant changes therefore may be direct or in the reverse direction, depending on the parent type employed in the given study.

The observed changes in this species of bacteria are thus comparable with those which are noted in Drosophila where the mutations may be direct from the wild type to a mutant or in the reverse direction.

The data on A14 as found in table 2 are not very extensive. There are, however, two points of significance. The ratio of mutant to normal cells found in A14 is twice the ratio found in S15. Of 156,000 colonies observed without treatment 18 mutations were found (0.0116 percent). For S15, where ten times this number of colonies were observed, 92 mutations were found (0.0061 percent).

Again in A14, as with S15, it was found that multiple mutation occurred much more frequently than expected. Of the 25 mutant colonies observed after X-ray treatment of the A14 stock, four exhibited mutation in two characters. In the spontaneous groups all 18 mutants had changed in one character only.

MUTATION AND VIRULENCE

Several of the X-ray-induced variants showing pronounced morphological changes were tested for a possible change in virulence associated with the morphological change on which the mutant was classified. These mutants were tested on susceptible maize seedlings (Inbred GB797), at the growth stage when two to three leaves had expanded on the seedling, by inoculating hypodermically through the first node with a heavy suspension of the test bacteria. Green weights of each group of plants were taken 14 days after inoculation and virulence index calculated.

$$100 - \frac{\text{Green weight of test plants} \times 100}{\text{Green weight of check plants}}$$

The indices may vary from zero to 100, the index increasing as virulence of the bacteria increases. The virulence indices for the different mutants tested are given in Table 6. All strains produced definite lesions and typical symptoms of bacterial wilt.

These mutants were derived from the two parental stocks S15 and A14 as indicated. These stocks were widely different in virulence as well as in the characters noted in the previous table. The virulence index for S15 was 31 and that for A14 was 75. Most of the mutants of the weakly virulent S15 stock exceeded the parent culture in virulence, although they did not equal the virulent culture (A14) in this respect. In contrast, most of the mutants of the highly virulent A14 were less virulent than the parent but more so than the weakly virulent S15 culture. These results may be explained by the fact that each parent is an extreme type and only mutations away from the extreme can be detected easily. It may be that when the gene combination is such as to cause extremely low virulence, the

probability of a change toward increased virulence is greater than a change toward still lower virulence. With a highly virulent organism, such as A14, the converse is true.

There are certain apparent correlations of colony morphology and virulence (table 6). The change from a rough to a smooth form seems to favor an increase in the invasive power of the organism. In the S15 (400) series,

TABLE 6

Virulence indices of mutants

SOURCE AND CULTURE NUMBER	COLONY CHARACTERISTICS	VIRULENCE INDEX
From S15 stock		
Parent (400)	Dark yellow, Rough I	31
476	Pale yellow, Rough I	35
420	White, Rough I	28
427	Rough II	42
417	Rough III	30
457	Rough IV	45
429	Rough VI	49
441	Smooth	58
452	Smooth	62
467	Smooth	41
431	Smooth	70
479	Smooth, mucoid	56
458	Small	28
From A14 stock		
Parent (500)	Large smooth, undifferentiated center	75
502	Large smooth differentiated center	81
506		73
560	Small smooth compact dry	65
520	Small smooth compact dry	51
530	Small smooth compact dry	53
525	Rough mucoid	57
526	Rough	46
537	Small S, non-mucoid	53
509	White	78

smooth variants are more virulent than the rough parent or the rough variants. In the A14 (500) series, the large colony smooth variants retain the virulence of the parents, and the small compact dry type or the more rough colonies are less virulent. It is evident that mutations in morphology may be attended by either increase or decrease in virulence and that such changes are not uniformly in the direction of reduced or increased pathogenicity.

STABILITY OF MUTANTS

The mutation rate of observed variants has not been determined except in a preliminary way. An indication of the stability of the variants, however, is obtained from their behavior when kept as stock cultures. When they first were observed, the mutants were plated and their purity checked; they were then placed on nutrient dextrose agar slants and kept in a refrigerator at 7°C. These stock cultures were transferred every six weeks. When plated again after nine transfers, most cultures were pure or predominantly pure for the original variant type. A few cultures contained variants, but in most cases the predominant type was that of the original. It should be noted, however, that the number of colonies that were observed in these ninth transfer platings were not large, usually fewer than 750 per culture.

The unstable types or segregating variants are more difficult to hold as stock cultures because they also form one or more stable types which usually become predominant and replace the unstable variants. The eventual predominance of the stable type is to be expected, for this type is not only reproducing itself but is being added to by reproduction of the unstable type.

DISCUSSION

The variations in *Phytomonas stewartii* described in this paper were obtained by examining individual colonies, almost all of which had arisen from single cells. The cell originating these variants arose under the stimulus of X-rays of low quantum energy. Under the conditions of treatment used the intensity was such that 100,000,000 viable cells were reduced to about 1,000 in 25 minutes of irradiation. The types of variants that arose under the two treatments were similar, and these in turn were similar to the spontaneous variation that occurs during growth. Thus under both natural and X-ray conditions the following changes have been observed: in colony type from rough to smooth and from smooth to rough, from one rough colony type to another, from one smooth colony type to still another, from the mucoid colony type to the non-mucoid and the reverse, in alteration of the pigment intensity of the parent type to that of greater or lesser intensity for the variant, and in increased or decreased virulence. Such variations frequently are spoken of as dissociation and are considered by some to be one phase of a cyclogenic type of life cycle (HADLEY 1927). Since X-irradiation resulted in a much higher rate of mutation, we can be relatively certain that the variation stimulated by X-irradiation is mutation as commonly understood. Therefore, it appears logical that similar but spontaneous variation be likewise considered as

mutation. The terms "dissociant," "saltant," "variant," and "mutant" on this evidence might be considered synonymous and due to hereditary changes resulting from gene mutation.

Increasing the rate of mutation is the most striking effect of X-rays on variability. Except for mutations to an unstable form, the mutations are stable and heritable from parent to daughter cells and therefore must be considered direct changes in the germ plasm. Such variants have proven stable in a variety of environments and for many cell generations.

In addition to the effect of X-rays in initiating mutation, they also cause inactivation or death of the bacterial cell. Increasing the dosage of X-rays of all quantum energies increases the number of inactivated bacterial cells. Similarly, increasing the dosage also increases the rates of mutation for different phenotypic characters. It seems entirely probable that the differences between inactivation and mutation are only those related to the position in which the energy is absorbed by the genic substances. Since most genes are necessary for life, the destruction of the capacity of a gene to reproduce would in effect eliminate that gene, and if the organism were haploid, the individual. Similarly, if the energy were absorbed by a chemical bond not primarily important to reproduction, the gene structure might be altered, thus leading to a visible mutation without lethally affecting the reproductive capacity of the cell.

Considerable evidence secured from this study indicates a basic similarity in the mechanism and the physical basis of inheritance between groups as widely divergent as *Ph. stewartii* and *Drosophila melanogaster*. The mutation rates observed for *Ph. stewartii* are of about the same order of frequency on a roentgen basis as the mutation rate in Drosophila. Certain differences in the frequency of appearance of the different mutations in the spontaneous and X-ray groups of *Ph. stewartii* further suggests this similarity. After X-ray treatment, unstable mutants have a much greater frequency than they do in the spontaneous group. This fact corresponds to a similar observation in Drosophila where X-rays markedly increase the unstable mutants as compared with stable mutants. These variegated types frequently are associated with breakage of the gene-bearing material, the chromosomes often being reorganized in a complex form. The observed increased frequency of such types in the irradiated bacteria suggests the possibility that in this form as in higher organisms there is some organization of the genic substances into larger entities comparable to chromosomes.

The spontaneous mutation rate of *Ph. stewartii* indicates a stability of the species comparable with that of the higher forms. Variants, when they appear, arise suddenly and in their final form. Variation appears to be

abrupt and discontinuous with no series of gradual changes between the parental type and that of the variant. This is equally true of the X-ray changes.

The individual characters in these bacteria generally mutate singly as they do in Drosophila. However, mutations of two or three characters in the same individual have been observed, and at a greater frequency than expected by chance in both the spontaneous and X-ray material. It has been argued that such multiple changes in Drosophila genes when exposed to X-rays are brought about by the particular direction the photoelectron takes through the inheritance substance. If the path traverses two or more genes, a double mutation would be produced. For these bacteria this argument would seem to be invalid, since we find that the relative frequency of these double changes is greater in the spontaneous mutation group than in those treated with X-rays. No explanation of this relationship is available at the moment.

The observed variation is classified readily into the broad groups of colony color, colony surface, or colony size. Within these groups, however, there are other distinct types. For example, in the S15 study, five rough types and three intensities of yellow pigmentation have been observed. The series of rough mutants pictured in both Plate I (A, B, C, D, E) and in Plate II (L, M, N) gives the impression of a graded series as does the colony color series—dark yellow, pale yellow, and white. A possible series of smooth mutants is not clear cut, but the impression gained from work with mutants of this species is that there is a series of almost infinitely small differences from a uniformly non-chromogenic smooth type to a uniformly chromogenic type. The intermediate types of this series are initially chromogenic and with later growth of the colony form non-chromogenic material around this center (see "smooth" colonies Plates I and II). Smoothness and roughness may be a distinction indicating different stages of the same series. The similarity of these described series to the classical allelic gene series of Drosophila eye color, rodent coat color, and anthocyanin color in maize or primula is striking, but since similar series can be arranged for characters due to non-allelic genes, it cannot of itself be considered proof of allelism.

Most mutations of morphological characters observed in this study were associated with certain changes of function. This is evidenced by the fact that the virulence of the mutants caused by X-rays may be increased or decreased. It is of interest to note, however, that the virulence of the weakly pathogenic strain used in this study was more frequently increased than decreased by mutation, whereas the virulence of the highly pathogenic strain was more frequently decreased than increased. The observed series of discontinuous changes in virulence shows that definite genes con-

trol the pathogenicity of *Ph. stewartii* for maize. With this species McNEW (1940) and LINCOLN (1940) have described rather complete series of virulence change that range from avirulence to high virulence. Such series may be the expression of multiple alleles, of multiple factors, or interaction of some other type.

The evidence herein presented indicates strongly a strict gene basis for heredity in the bacteria as in other organisms. As is well known, this inheritance affects many physiological as well as color or morphological characters. Strains differ in their disease causing ability or enzyme forming capacity for the fermentation of sugars or breaking down of proteins, etc. The work herein presented favors the view that each of these changes is controlled by individual genes.

SUMMARY

This paper presents a comparative study of mutations in *Phytomonas stewartii* under natural conditions and under the stimulus of X-irradiation of low quantum energy. Irradiation was at an intensity such that 100,000,000 viable cells suspended in broth were reduced to about 1,000 in 25 minutes of treatment. Survival during irradiation follows the simple exponential function—survival ratio $= e^{-ar}$. Mutations were observed in colony color, surface appearance, and size. The rate of mutation following X-irradiation is greater than the observed spontaneous rate. X-ray-induced mutation differs from spontaneous mutation only in the frequency of occurrence, no differences in the kind of mutation produced being observed. The pattern of mutation observed in two widely different stocks of *Ph. stewartii* was similar. Mutations of colony characters may be accompanied by either increased or decreased virulence for maize. Except for mutations to an unstable form, mutants appear to be as stable as the parent strain from which they were derived. From evidence secured in this study it would appear that the terms "mutant," "variant," "saltant," and "dissociant" as applied to bacteria are synonymous and are applied to phenomena resulting from gene mutation. From this point of view the physical basis of inheritance of *Ph. stewartii* is similar to that of higher organisms.

LITERATURE CITED

DUGGAR, B. M., 1936 Effects of radiation on bacteria. In Biological Effects of Radiation. Vol. II, 1119–1149. New York: McGraw Hill.

FRYER, H. C., and J. W. GOWEN, 1942 An analysis of data on X-ray-induced visible gene mutations in *Drosophila melanogaster*. Genetics **27**: 212–227.

HADLEY, P., 1927 Microbic dissociation. J. Infect. Dis. **40**: 1–312.

HABERMAN, S. and L. D. ELLSWORTH, 1940 Lethal and dissociative effects of X-rays on bacteria. J. Bact. **40**: 483–503.

JOHNSTON, O., and A. M. WINCHESTER, 1934 Studies on reverse mutations in *Drosophila melanogaster*. Amer. Nat. **68**: 315–358.

LANGE, L., and M. FRAENKEL, 1923 Die Wirkung von Röntgenstrahle auf Tuberkelbazillen. Klin. Wochensch. **2**: 1161–1162.

LINCOLN, R. E., 1940 Bacterial wilt resistance and genetic host-parasite interactions in maize. J. Agric. Res. **60**: 217–240.

LINCOLN, R. E., and J. W. GOWEN, 1942 A test for sexual fusion in bacteria. In press.

MCNEW, G. L., 1938 Dispersion and growth of bacterial cells suspended in agar. Phytopathology **28**: 387–401.

1940 Factors influencing attenuation of *Phytomonas stewartii* cultures. J. Bact. **39**: 171–186.

PINNEY, L. E., 1939 A gas-type X-ray tube. Iowa State Coll. J. Sci. **13**: 269–273.

TAYLOR, L. S., and G. SINGER, 1930 An improved form of standard ionization chamber. Bur. Stand. J. Res. **5**: 507–516.

TIMOFEEFF-RESSOVSKY, N. W., 1933 Rückgenmutation und die Genmutabilitat in verschiedenen Richtungen. IV. Röntgenmutationen in verschiedenen Richtungen am white-locus von *Drosophila melanogaster*. Z. i. A. V. **65**: 279–292.

1937 Mutationsforschung in der Vererbungslehre. Dresden und Leipzig: Theodor Steinkopff.

WYCKOFF, R. W. G., and J. R. LAGSDIN, 1930 Simple self-rectifying gas X-ray tube. Radiology **15**: 42–48.

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, of cytology, taxonomy, embryology, physiology, biometry, or mathematics, if of sufficient importance and of such a character as to be of primary interest to the geneticist. For the present, the length of manuscripts will be limited to twenty-five printed pages (about twelve thousand words) except by special vote of the Editorial Board. Tabular matter in excess of one-fourth of the manuscript can not be printed, unless of particular importance, but will be kept on file for reference on request provided two copies are furnished by the author. Excess pages will be printed if paid for by the author (about six dollars a page).

Manuscripts are printed, ordinarily, in the order of their receipt. They may be printed out of turn provided the entire cost is paid by the author. Such material will be added to the current number and will not delay the publication of any other article.

Contributors are requested to use care in the preparation of manuscripts. Carbon copies can not be considered. All references to literature should cite the name of the author, followed by the year of publication, the papers so referred to being collected into a list of "LITERATURE CITED" at the end of the article. In this list care should be taken to give the titles in full, and to indicate accurately, in Arabic numerals, the volume number, the first and last pages, and the date of publication of each paper if published in a periodical, and the number of pages, place and date of publication, and the name of publisher, of each independent publication. The arrangement of this list should be alphabetical by author and chronological under each author. Titles of publications are abbreviated according to the *World list of scientific periodicals*, Oxford University Press, London and New York, 1925.

Each manuscript should include a summary of the evidence and of the conclusions. Factor symbols should be separated so that they can be properly identified and underlined for italics. Gene symbols with sub- or superscript letters should be avoided except in long allelic series. Most typewriters do not distinguish between the letter l and the figure 1 or the hyphen and the dash. Such distinctions should be made wherever there is a possibility of confusion.

Footnotes should be avoided wherever possible. Usually, they can be enclosed in parentheses and inserted after the sentences to which they apply. If used in the text they should be numbered consecutively in a single series and designated by Arabic superscript numerals. Footnotes to tables should be marked with an asterisk, dagger, or other symbol so as not to be confused with the figures in the tables.

Illustrations should be referred to as figures wherever possible. Plates are reserved for illustrations that require paper inserts or for collections of small figures that can not be designated conveniently as separate figures. Text figures should be clearly identified but not numbered in the illustration. Figures included in plates should be distinguished by letters rather than by numbers. All figures and plates are reduced to a maximum of $4\frac{3}{8}$ inches in width and 6 inches in height.

Legends for figures and plates should be typewritten separately from the illustrations for the reason that the type is set by the printer and the illustrations are made by the engraver. Mistakes are likely to occur if the separation is not made by the author.

Galley proofs and, whenever there is time, page proofs will be sent. Authors should leave forwarding directions whenever they are to be away from the address sent with the manuscript. Ordinarily page proofs can not be sent out of the country. Both proofs must be returned promptly and no extensive change may be made in page proofs, which is not compensated for within the same paragraph, or in an adjacent paragraph on the same page. Changes from copy will be charged to the author.

GENETICS furnishes 75 reprints, without covers, free. Covers and additional reprints may be secured at actual cost of manufacture provided these are ordered when the corrected galley proofs are returned.

Manuscripts and all editorial correspondence should be addressed to the Editor of GENETICS, 704 Schermerhorn Hall, Columbia University, New York.

GENETICS Vol. 27 No. 4 JULY, 1942

CONTENTS OF PRECEDING ISSUES

SEPTEMBER, 1941

EMERSON, STERLING, Linkage relationship of two gametophytic characters in *Oenothera organensis*.

HARMAN, MARY T., and ANNETTE ALSOP CASE, Genetic aspects of pigment production in the guinea pig.

BRINK, R. A., and D. C. COOPER, Incomplete seed failure as a result of somatoplastic sterility.

NEEL, JAMES V., A relation between larval nutrition and the frequency of crossing over in the third chromosome of *Drosophila melanogaster*.

STURTEVANT, A. H., and E. NOVITSKI, The homologies of the chromosome elements in the genus Drosophila.

MCCLINTOCK, BARBARA, The association of mutants with homozygous deficiencies in *Zea mays*.

NOVEMBER, 1941

GOODRICH, H. B., G. A. HILL, and MYRON S. ARRICK, The chemical identification of gene-controlled pigments in Platypoecilus and Xiphophorus and comparisons with other tropical fish.

KIKKAWA, HIDEO, Mechanism of pigment formation in Bombyx and Drosophila.

RANDOLPH, L. F., Genetic characteristics of the B chromosomes in maize.

GILES, NORMAN, Spontaneous chromosome aberrations in triploid Tradescantia hybrids.

WRIGHT, SEWALL, Tests for linkage in the guinea pig.

Index to Volume 26.

JANUARY, 1942

Portrait of J. ARTHUR HARRIS.

LINDEGREN, CARL C., and GERTRUDE LINDEGREN, Locally specific patterns of chromatid and chromosome interference in Neurospora.

BRADLEY, J. O., Meiotic chromosome behavior in species, species hybrids, haploids, and induced polyploids of Gossypium.

CLELAND, RALPH E., The origin of *decipiens* from the complexes of *Oenothera lamarckiana* and its bearing upon the phylogenetic significance of similarities in segmental arrangement.

STADLER, L. J., and FRED M. UBER, Genetic effects of ultraviolet radiation in maize. IV. Comparison of monochromatic radiations.

HINTON, TAYLOR, A comparative study of certain heterochromatic regions in the mitotic and salivary gland chromosomes of *Drosophila melanogaster*.

Abstracts of papers presented at the 1941 meetings of the Genetics Society of America.

MARCH, 1942

CUMLEY, R. W., and M. R. IRWIN, Immunogenetic studies of species: Segregation of serum components in backcross individuals.

JENNINGS, H. S., Genetics of *Paramecium bursaria*. III. Inheritance of mating type, in crosses and in clonal self fertilizations.

FRYER, H. C., and JOHN W. GOWEN, An analysis of data on X-ray-induced visible gene mutations in *Drosophila melanogaster*.

IRWIN, M. R., and R. W. CUMLEY, Immunogenetic studies of species: Qualitative differences in the serum of backcross progeny following a generic cross in birds.

STALKER, HARRISON D., Sexual isolation studies in the species complex *Drosophila virilis*.

DUNN, L. C. Studies of spotting patterns. V. Further analysis of minor spotting genes in the house mouse.

MAY, 1942

KIMBALL, R. F., The nature and inheritance of mating types in *Euplotes patella*.

PIPKIN, SARAH BEDICHEK, Intersex modifying genes in wild strains of *Drosophila melanogaster*.

LEONARD, WARREN H., Inheritance of fertility in the lateral spikelets of barley.

EPLING, CARL, and TH. DOBZHANSKY, Genetics of natural populations. VI. Microgeographic races in *Linanthus Parryae*.

ATWOOD, SANFORD S., Oppositional alleles causing cross-incompatibility in *Trifolium repens*.

CHASE, HERMAN B., Studies on an anophthalmic strain of mice. III. Results of crosses with other strains.

REED, S. C., C. M. WILLIAMS, and L. E. CHADWICK, Frequency of wing-beat as a character for separating species races and geographic varieties of Drosophila.

Conservation of Scholarly Journals.

SINGLE NUMBERS $1.25 ANNUAL SUBSCRIPTION $6.00

obtainable from

BROOKLYN BOTANIC GARDEN, BROOKLYN, NEW YORK